Culture and Management of Sturgeon and Paddlefish Symposium Proceedings
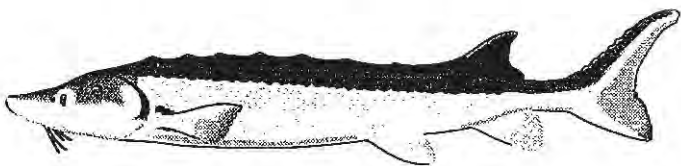
Serge Doroshov
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International Congress on the Biology of Fishes

San Francisco State University July 14-18, 1996

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Sturgeon and Paddlefish

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Physiology Section $\mathbf{A}\mathbf{F}$ $\mathbf{S}$ *American Fisheries Society*

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PREFACE

These proceedings contain extended abstracts of most of the papers presented during the Sturgeon and Paddlefish Symposium held at the International Congress on the Biology of Fishes. The International Congress was sponsored by the Physiology, Fish Culture and Fisheries Management Sections of the American Fisheries Society and was held at San Francisco State University, San Francisco, California. The majority of submitted papers deal with the North American species, but several interesting papers on the Eurasian sturgeons are also included.

Sturgeon and paddlefish (Acipenseriformes) occupy a special place in fish biology, aquaculture, and comparative zoology. Their phylogeny, lifecycles, and history of exploitation by man are unique. In the past, they played a significant role in commercial fisheries. Despite dramatic declines in abundance, they continue to attract commercial and sport fishermen due to the high value of their meat and roe and their qualities as a game fish. In spite of the delayed sexual maturity of these species, aquaculturists have succesfully domesticated paddlefish and several sturgeon species. In the U.S., sturgeon aquaculture is becoming a major producer of meat and roe for human consumption.

The potential phylogenetic origin of Acipenseriformes from the common ancestor of both the ray-finned fishes and the tetrapods makes living species of sturgeon and paddlefish highly attractive for comparative research in vertebrate evolution. Previously, scientists encountered great difficulty in obtaining these species for studies. However, sturgeon and paddlefish are becoming increasingly available and attract researchers worldwide. During the past 20 years, significant new knowledge has been gained concerning sturgeon population biology, ecology, physiology, pathology, nutrition, and genetics.

Several factors contributed to the recent progress in sturgeon research, particularly in western countries: a rapid development of aquaculture and hatchery technology for many sturgeon species; growing public awareness of the unique value of these "living fossils" and the need for their protection; an emergence of new research tools (e.g. telemetry, computer modelling, molecular biology) which are effectively used in field and laboratory studies. Finally, the recent geopolitical changes and modern communication technology have opened opportunities for information exchange with the scientists from many eastern countries.

International interest to sturgeon and paddlefish appears to have grown exponentially. In 1983, a Symposium on the North American Sturgeons was held in conjunction with the annual meeting of the American Fisheries Society at Milwaukee, Wisconsin. Since that meeting three additional symposia and conferences were held in France (Bordeaux, 1989), Russia (Moscow, 1993) and in the U.S. (New York, 1994) which all focused on sturgeon biology, aquaculture, and conservation. The next Symposium is expected to be in Italy (Piacenza, July 1997), which is one of the leading countries in development of sturgeon aquaculture and conservation biology.

In spite of recent advances in the culture and management of Acipenceriformes, the current state of the world sturgeon and paddlefish resources is not a rosy picture. Several species may be lost beyond recovery to unregulated fishery, river damming, and man-induced ecological catastrophes

(such as one in the Aral Sea). It is our duty, as fish biologists and aquaculturists, to continue our efforts in conservation, protection, and propagation of sturgeon and paddlefish resources worldwide.

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CONGRESS ACKNOWLEDGEMENTS

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COLUMBIA RIVER WHITE STURGEON

INVESTIGATIONS DOWNSTREAM OF

HUGH L. KEENLEYSIDE DAM, BRITISH COLUMBIA

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Abstract.—White sturgeon (*Acipenser transmontanus*) in the lower Columbia River in British Columbia, Canada were investigated from 1990 to 1995 by R.L. & L. Environmental Services Ltd. The white sturgeon population was localized in their distribution and predominantly restricted to four main (high use) areas of the mainstem Columbia River. These areas were characterized by low (0.1 m/s) to moderate (0.4 m/s) water velocities, depths that ranged from 8 to 50 m, and substrates comprised mainly of finer bed materials. The high use areas were associated with either the confluence areas of major tributaries, dam tailwaters, or deep eddy pools. White sturgeon utilized these preferred habitats for extended periods of time and generally exhibited only localized movements within and between adjacent high use areas. The population was estimated at approximately 1100 white sturgeon and consisted mainly of individuals over 1.0 m in total length (TL). A substantial shift in size-class composition from a population dominated by fish under 1.0 m (TL) in the early 1980s to the present population comprised primarily of fish over 1.0 m (TL) was attributed to a lack of recruitment. The dynamics of the population were within the ranges reported for white sturgeon studies conducted on southern populations in the U.S. Spawning has been confirmed annually since 1993 at only one of several potentially suitable spawning sites available in the study area. Available evidence suggests this area may provide the most suitable spawning conditions for a population that annually may have only a limited number of potential female spawners.

Introduction

Since 1990, B.C. Hydro has commissioned investigations of white sturgeon populations in the Canadian portion of the Columbia River below Hugh L. Keenleyside Dam (HLK). The results of preliminary inventory studies in 1990 and 1991 indicated the presence of localized concentrations of white sturgeon (*Acipenser transmontanus*) in the study area. The low abundance of juvenile white sturgeon in the catch suggested recruitment of white sturgeon in the region may be limited, possibly due to the presence and operations of dams on the mainstem Columbia River. To obtain additional information on existing white sturgeon populations in the Columbia River below HLK, further studies were conducted from 1992 to 1994 at the request of B.C. Hydro. On the basis of results

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obtained during these studies and the assignment of a "Red Listed - S1" (i.e., critically imperilled or endangered) status by the B.C. Conservation Data Centre (1996), additional studies were commissioned in 1995 to monitor the status of white sturgeon in the area.

The specific objectives of the studies were to obtain information on white sturgeon distribution and population dynamics, identify and typify important sturgeon habitats, assess movement patterns, and determine the occurrence and success of spawning. The study area included the 56 km of the mainstem Columbia River from the Hugh L. Keenleyside Dam to the Canada-U.S.A. border and the lower reaches of major tributaries.

Prior to 1992, regulations allowed for the annual harvest (per angler) of one white sturgeon over 1.0 m in total length (TL). In 1992, a slot limit was imposed on the fishery that allowed an annual harvest (per angler) of one white sturgeon between 1.0 m and 1.5 m TL. Since 1993, the fishery was designated as catch-and-release and in April 1996, the fishery was closed to angling.

Methods

White sturgeon were collected using a variety of capture methods in an attempt to collect a wide range of age-classes from different habitat types (R.L. & L., 1994). Set lines were the primary method used to sample white sturgeon. D-ring drift nets and artificial substrate mats were employed to capture egg and larval stages of white sturgeon. Habitat data collected at fish, egg, and larvae capture locations included water temperature, depth and velocity, substrate type, and flow patterns.

White sturgeon were collected during the spring, summer and fall periods to acquire seasonal information on relative abundance, sex ratio, maturity, growth, condition, habitat use and movements. Captured white sturgeon were marked with both T-anchor tags and PIT tags. White sturgeon were surgically examined to determine sexual maturity stage. Mature fish that would likely spawn within the following year were equipped with externally mounted radio transmitters. Population estimates were derived from the mark-recapture data. White sturgeon were weighed (kg) and measured for fork length (cm), total length (cm), and girth (cm). A section of the left pectoral fin ray was collected for ageing purposes. Rate of growth was described using a von Bertalanffy growth model.

Figure 1.—Study area for Columbia River white sturgeon investigations.

Results and Discussion

Distribution

White sturgeon in the 56 km section of the Columbia River below Hugh L. Keenleyside Dam (HLK) in Canada have a localized distribution, tend to exhibit mainly localized movements, and select deep water habitats in the upper and lower sections of the study area (Figure 1). Four areas were assigned a high use rating based on the consistent presence of white sturgeon during all seasons. White sturgeon use of these areas reflected the

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availability of riverine habitats with lower velocity relative to average mainstem flows and depths that exceeded 15 m. Three of the four primary holding/feeding areas were associated with major inflow sources (i.e., tributary confluences or dam tailwaters). The importance of these areas to white sturgeon is magnified considering the small fraction of the total riverine habitat these areas represent. Alterations to the suitability of these habitats would have substantial impacts on white sturgeon populations.

A total of 1341 white sturgeon have been sampled in the Columbia River study area. Of these, 870 individuals have been marked since 1990; 387 (44.5%) have subsequently been recaptured. Recaptured percentages of marked fish increased from 4.3% in 1990 to 52.7% in 1995.

Catch-rates of white sturgeon provided an indication of presence and relative abundance between areas within the same sample period, but were not a reliable indicator of seasonal or annual population abundance. As a result of the variability in catch rates between and within sample years, consistent trends in abundance were not evident at any of the high use areas. This variability may reflect: (1) fluctuations in white sturgeon density due to movements in or out of high use areas, (2) seasonal differences in feeding activity, possibly influenced by discharge or water temperature, or (3) the relative efficiency of the sample gear at different discharge regimes. R. L. & L. (1994) reported a significant negative correlation between discharge and catch rate that suggested effects of discharge on feeding activity or gear efficiency were partly responsible for the observed variation in white sturgeon catches. Whether the mechanisms that produced this correlation were related to the physical effects of discharge, differences in feeding activity, or differences in gear efficiency remains unclear.

Movements

Fifty white sturgeon have been equipped with radio transmitters since 1990. Of the 36 white sturgeon that retained functional transmitters for at least one full year, 39% exhibited net movements of less than 5 km, 25% moved between 5-10 km, and 36% moved more than 10 km (Figure 2). These percentages differed from those recorded for the 386 conventionally tagged white sturgeon recaptured in the study area, where 93% moved less than 5 km, 4% moved from 5-10 km, and 2% moved more than 10 km between their release and recapture locations (Figure 2). The difference in movement between radio tagged and conventionally tagged white sturgeon was attributed to the maturity class composition of the two groups. The radio-tagged fish were mainly mature or pre-spawning fish while the conventionally tagged group consisted mainly of immature or non-spawning adults. The low incidence of movements greater than 10 km for both tagged groups suggests white sturgeon exhibit lengthy resident times within localized areas.
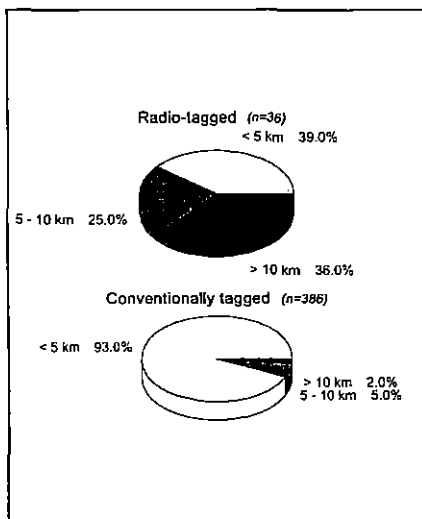
Figure 2.—Percent frequency of distances moved by white sturgeon in the Columbia River, 1990 to 1995.

White sturgeon movement patterns in the Columbia River study area are not entirely understood. Defined migrations of white sturgeon into or out of the areas were not identified. Localized

movements were commonly recorded within the study area and fish occasionally moved between high use areas in the upper and lower sections. Some fish moved downstream into U.S. portions of the river. Most white sturgeon exhibited localized movements either within or between adjacent high use areas. These movement patterns are similar to those described for white sturgeon populations in the U.S. (Haynes et al., 1978; Apperson and Anders, 1991; Brannon and Setter, 1992).

White sturgeon exhibited movements out of deep areas to nearby shallow water habitats during the spring to summer period. These movements were attributed to feeding activities since this period coincides with the highest seasonal abundance of prey species in near-shore habitats within the mainstem Columbia River.

Population Dynamics

Estimates of the white sturgeon population in the Columbia River study area in 1995 indicated a population of approximately 700 individuals in the upper section and 400 in the lower section for a total population of 1100 fish. This estimate primarily reflects the abundance of white sturgeon in the four high use areas in the upper and lower sections of the Columbia River. The abundance of white sturgeon in the middle section is low. The population in the U.S. portion of the Columbia River above Grand Coulee Dam is currently unknown. Limited available data suggest white sturgeon in Roosevelt Lake do not frequently intermix with the population in the Canadian portion of the Columbia River. The relationships of white sturgeon populations between Canada and U.S. sections of the Columbia River is an aspect of sturgeon life history that requires further investigation.

White sturgeon captured since 1990 have ranged in fork length from 61 to 271 cm. The length-frequency distributions were grouped into undersize, mid-size, and oversize classifications representing fish less than 1 m in total length (TL), between 1.0 and 1.5 m TL, and greater than 1.5 m TL, respectively. These size-classes roughly correspond to juvenile, sub-adult, and adult maturity stages of the population. Catches in all years were dominated by mid-size and oversize cohorts.

The length-frequency distribution of white sturgeon in the study area has shifted in the last decade from a population comprised predominantly of undersize fish in the early 1980s to one presently dominated by fish in the mid-size and oversize classes (Figure 3). Approximately 65% (n=82) of white sturgeon caught by angling in the 1980s were in the undersize category compared to 7% in 1990 and 10% from 1992 to 1995. The reported size distribution of white sturgeon in Roosevelt Lake (Brannon and Setter, 1992) was similar to that recorded below HLK which implies that high numbers of juveniles are not present in either area.

A survival rate of 0.79 was calculated for white sturgeon in our study. This was near the mid-range of survival rates presented for white sturgeon populations elsewhere in North America. While slot limits or harvest restrictions may provide significant short-term
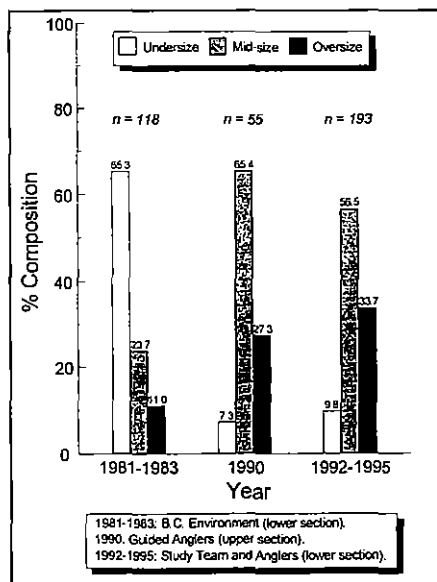
Figure 3.—Percent composition of white sturgeon size-classes in the Columbia River.

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benefits to white sturgeon populations, overexploitation to the point of stock collapse may not necessarily be prevented. Recruitment responses in the Columbia River may be less productive for fish segregated by dams. Exploitation of isolated stocks with limited recruitment would cause population collapse within a shorter period of time than for populations with stable recruitment. There is a concern that the natural recruitment of white sturgeon in the Columbia River between Hugh L. Keenleyside and Grand Coulee dams may currently be insufficient to maintain the existing population, even with the closure of the fishery.

A von Bertalanffy growth curve (von Bertalanffy, 1938) was developed for white sturgeon aged from the study area during the 1990 to 1995 period (Figure 4). The model provided a method that facilitated the most reliable and consistent comparisons of growth rates for populations with similar growth characteristics, assuming biases in age estimates from fin ray sections of different populations are similar. The growth model depicted a slower growing population compared to growth models for southern Columbia River populations (Kohlhorst et al., 1980; Brennan and Cailliet, 1989; Rieman and Beamesderfer, 1990; Beamesderfer and Rien, 1993; Tracy and Wall, 1993).
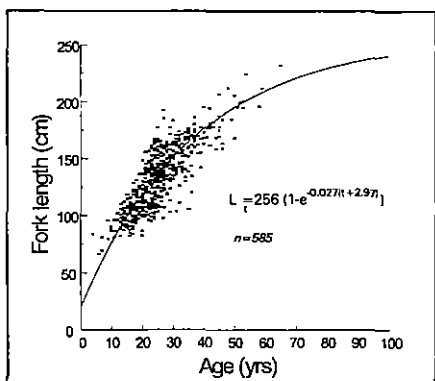
Figure 4.—von Bertalanffy growth curve for white sturgeon captured in the Columbia River, 1990 to 1995.

The equation shown in the figure:

$$L_t = 256 \left(1 - e^{-0.027(t + 2.97)}\right)$$

$$n = 585$$

Spawning

Potential spawning habitat for white sturgeon is available at several locations within the study area. Three of these sites are located in the tailrace areas of dams and four are located in high-velocity rapid sections of the mainstem Columbia River. Spawning has been confirmed at only one location, this being the Waneta Dam tailrace area at the confluence of the Pend d'Oreille and Columbia rivers. This area may represent the most suitable spawning location for sturgeon, although the occurrence of spawning at other potential areas cannot be dismissed based on the degree of sampling effort to date.

The Pend d'Oreille River exhibits a more natural hydrograph and warms more rapidly in the spring than either the Columbia or Kootenay rivers. This may be important if increasing discharge and temperature are critical environmental factors inducing vitellogenesis and providing cues for ovulation. In the Waneta area, water temperatures exhibited a gradual, sustained increase prior to and during the spawning period (Figure 5). In addition, parameters of water temperature, water depth, water velocity, and substrate in the Waneta area
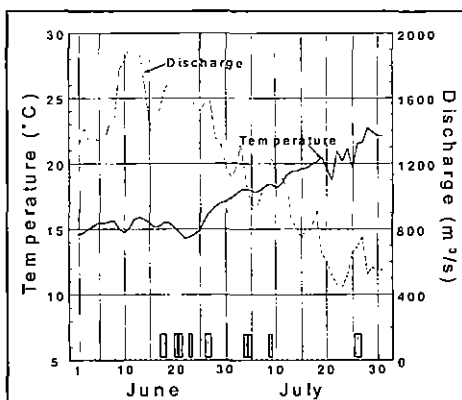
Figure 5.—Daily discharge from Waneta Dam and mean daily water temperatures of the Pend d'Oreille River during the white sturgeon spawning period in 1995. Shaded bars on horizontal axis denote spawning events.

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were all in the optimal suitability range determined for white sturgeon spawning in the lower Columbia River. The confined channel morphology of the Waneta area results in high water velocities over a wide range of discharges similar to the situation reported below Bonneville Dam in the lower Columbia River (Parsley and Beckman, 1994).

In 1995, nine distinct white sturgeon spawning events were identified in the confluence area of the Pend d'Oreille and Columbia rivers (Figure 5). The lack of evidence to indicate white sturgeon spawning in other potentially suitable habitats in the study area may suggest physical conditions are unsuitable to promote vitellogenesis or cue ovulation. At Waneta Dam, temperatures prior to and during the spawning period increased steadily and were within the optimal egg development range (14-16°C) during periods of above average discharge more consistently than at other potential spawning areas in the study area.

Spawning consistently occurred on the downward limb of the hydrograph over the June to July period and at water temperatures greater than 14°C. The 1995 hydrograph of the Pend d'Oreille River revealed numerous spikes in discharge that occurred during the spawning period (Figure 5). Spawning also was recorded during periods of fluctuating discharges that, in prior years, were the result of peaking operations at Waneta Dam. These results suggested daily or weekly fluctuations in flow may not preclude spawning, if the proper cues are present to stimulate spawning and sufficient discharge is provided to maintain velocities in spawning habitats above a critical level.

Several spawning events in the Waneta area have been recorded after water temperatures exceeded 18°C. White sturgeon eggs incubated at temperatures above 18°C exhibit an increased rate of mortality and abnormal development (Wang et al., 1985). Anders and Beckman (1993) recorded a high incidence of mortality (98%) for white sturgeon eggs spawned at water temperatures between 18 and 19°C below Dalles Dam on the lower Columbia River. These authors stated that spawning at these temperatures would not contribute to recruitment.

In 1995, four of the nine spawning events recorded in the Waneta area occurred when water temperatures exceeded 18°C (Figure 5). The loss of a substantial portion of the total annual egg deposition has serious implications to a population with limited reproductive potential.

Reasons for the high incidence of delayed spawning by white sturgeon in the Waneta area are unknown, but may be related to regulation of the Pend d'Oreille River. River regulation and disturbances within the basin (i.e., logging) may have increased the warming rate of the Pend d'Oreille River during the June to July period. Historically, water temperatures may have persisted in the 14 to 16°C range for greater periods that provided an increased duration of time over which successful spawning could occur. Another possible factor that may influence the timing of spawning is that pre-spawning females stage in the Columbia River that remains cooler and warms more slowly than the Pend d'Oreille River. When these females move into the lower Pend d'Oreille River to spawn, the temperature differential between the two systems is often in the 3-5°C range. If the timing of spawning is based on temperatures within the Columbia River then spawning in the Waneta area during elevated water temperatures may adversely effect egg development.

Conclusion

White sturgeon spawning has been confirmed in the Waneta area although evidence to indicate successful recruitment is lacking. A review of the literature indicates there are numerous factors that potentially could reduce the success of white sturgeon reproduction in the study area. The natural hydrologic and thermal regimes of the mainstem Columbia River, the Kootenay River, and the Pend d'Oreille River have all been altered by flow regulation. Study results to date indicate that these alterations may have reduced white sturgeon reproductive success in the Columbia River between Hugh L. Keenleyside Dam and Grand Coulee Dam. Similar instances of reduced

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reproductive success and poor recruitment have been reported for other white sturgeon populations isolated by dams in the lower Columbia River in the United States (Rieman and Beamesderfer, 1990).

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DYNAMICS AND POTENTIAL PRODUCTION OF WHITE STURGEON IN THE

UNIMPOUNDED LOWER COLUMBIA RIVER

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Abstract

The unimpounded lower Columbia River downstream from Bonneville Dam (LCR) supports the greatest abundance and density of white sturgeon (*Acipenser transmontanus*) reported in the species' range. High productivity of the population resulted from growth that was as good or better than reported for other populations, the highest mean relative weight reported for any white sturgeon population, and a relatively low median age (24 years) of first maturity for females (95% of females matured between 16 and 35 years of age). Estimated instantaneous natural mortality was 10%. Estimated instantaneous fishing mortality for age 12-17 year fish averaged 36% in LCR fisheries during 1986-1990. The 1986-1993 average annual abundance estimate of LCR white sturgeon ≥ 92 and ≤ 166 cm fork length (FL) was 124,100 fish at an average density of 2.0 fish per hectare. Population simulations, assuming constant recruitment, predicted a maximum sustainable yield (MSY) of 3.0 kg per recruit at an 18% exploitation rate (of the 82-166 cm FL population). Factors most responsible for the favorable production potential of the population were access to marine areas, abundant food resources, and consistently favorable hydrologic conditions during the spawning time frame which enhanced recruitment. Conservative management strategies combined with a high production potential have resulted in a healthy sturgeon population that supports one of the regions most valuable fisheries.

Introduction

The unimpounded lower Columbia River (LCR) supports one of the most productive sturgeon fisheries in North America and perhaps the world. Annual harvest of white sturgeon (*Acipenser transmontanus*) in the LCR has averaged 37,600 fish in recreational fisheries and 7,100 fish in commercial fisheries during the past ten years (ODFW and WDFW 1995). Average yield of white sturgeon in combined LCR fisheries during the past ten years has been approximately 400,000 kg annually. The sturgeon fishery ranks as the largest recreational fishery in the Columbia Basin in terms of effort with a ten year annual average of 158,000 angler trips (Melcher and Watts 1995).

The longevity, slow growth, and delayed maturation of sturgeon makes them susceptible to overexploitation (Rieman and Beamesderfer 1990; Rochard et al. 1990; Smith 1990; Birstein 1993). Excessive harvest in the 19th century collapsed Columbia River sturgeon stocks. Intensive sturgeon fishing on the Columbia River began in 1889 and peaked in 1892 with about 2,500,000 kg of sturgeon landed. The stock was depleted by 1899 after a ten year period of unregulated exploitation (Craig and Hacker 1940) (Figure 1). Season, gear, and minimum size restrictions failed to bring about an increase in sturgeon production as evidenced by poor yields during the first half of this century. Only after a maximum size regulation designed to protect sexually mature sturgeon was enacted in 1950 did the

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sturgeon population rebound. Annual harvests doubled by the 1970's and doubled again by the 1980's. Current harvest restrictions may be inadequate to protect stocks from overexploitation.

Sturgeon have been severely affected by hydroelectric development and operation which has drastically altered the large river systems they inhabit. Many species of sturgeon and paddlefishes are now endangered, threatened, or extinct due, in large part, to the destruction of their habitats (Rochard et al. 1990; Birstein 1993). Hydroelectric development of the Columbia River mainstem since 1933 may have reduced the productivity of white sturgeon (Fickeisen 1985; Beamesderfer et al. 1995). Pre-impoundment conditions can now be found only in the 234 km free-flowing reach downstream from Bonneville Dam. We believe the larger yields in lower Columbia sturgeon fisheries are evidence of a relatively high population productivity linked to the available habitat left unaltered in this reach of the Columbia.

Productive populations and high yield fisheries for white sturgeon can only be sustained through scientific management based on a thorough understanding of population dynamics and limiting factors. In this paper, we examine the characteristics of the white sturgeon population in the free-flowing LCR between the Pacific Ocean and Bonneville Dam located at river kilometer (rkm) 234 (Figure 2). We use this information to project sustainable yield and exploitation rate, and compare the characteristics of populations in the free-flowing LCR with impounded populations to help identify factors limiting production.
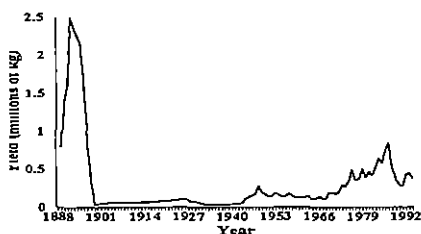
Figure 1. Annual yield (kg) of white sturgeon in the lower Columbia River, 1881-1994.
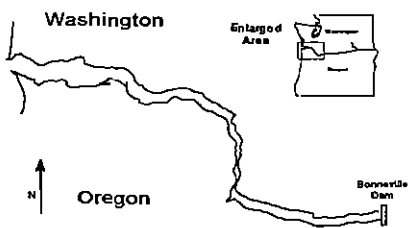
Figure 2. The unimpounded reach of the lower Columbia River downstream from Bonneville Dam.

Methods

Age, Growth, and Condition. - White sturgeon were aged by counting periodic rings on cross sections of the anterior pectoral fin ray (Rien and Beamesderfer 1994; Tracy and Wall 1993). Samples were obtained from consumptive and research fisheries during 1987-1992. Length measurements were made for each fish that had a fin ray sample removed. Length at age relationships for white sturgeon were quantified by fitting length (cm FL) and age (years) data to a von Bertalanffy growth function (VBGF). Aging techniques were validated using oxytetracycline (OTC) marking (Leaman and Nagtegaal 1987; Tracy and Wall 1993; Rien and Beamesderfer 1994). Two measures of accuracy of OTC ages were calculated: 1) the average net ageing error, defined as the sum of the signed differences between OTC ages, assigned by five experienced interpreters, and the actual winters at large divided by the number of readings and; 2) the correspondence ratio, defined as the percentage of OTC ages that matched the actual winters at large. Both measures were computed using assigned ages from all interpreters combined.

Length and weight (kg) measurements were taken during research fisheries, commercial and recreational fishery sampling, and from natural mortalities. Data were fitted to an exponential function using non-linear least squares regression. Relative condition was determined by estimating

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mean relative weight based on the standard weight determined for white sturgeon ($W_s = 2.735E\text{-}6$ $FL^{3.232}$) (Beamesderfer 1993). This analysis was restricted to FLs ≥ 54 cm to minimize effects of errors from developmental changes in body form from juveniles to adults (Beamesderfer 1993).

Reproductive Potential.- White sturgeon sampled in the commercial harvest (110-166 cm FL) were measured to the nearest cm FL and sexed by visual examination of gonads. Sturgeon >166 cm FL examined during broodstock monitoring activities were sexed by first trying to express sperm from ripe males. Females and males not fully mature were sexed by surgical biopsy according to the procedures outlined in Conte et al. (1988). Ovarian samples were preserved in 10% formalin.

Stage of maturity for females was categorized according to a qualitative histological classification modified from Chapman (1989). Ovarian maturity was classified into three general categories: immature/resting (all pre-vitellogenic fish, and early vitellogenic fish recovered from July to December), maturing (early vitellogenic fish recovered from January to June, and late vitellogenic fish recovered from April to December), and mature (ripe fish, spent fish, and late vitellogenic fish recovered from January to March). These classifications correspond to the estimated year of spawning: immature/resting fish would spawn ≥ 2 years from the recovery year, maturing fish would spawn the following year, and mature fish would spawn that year. The proportion of maturing females relative to the total sample of immature and maturing fish was estimated for each size class. A relationship describing size specific maturity was developed using a maximum likelihood estimation procedure which used a cumulative normal probability curve as a functional model of the maturation process (Welch and Beamesderfer 1993).

Exploitation and Mortality.- Annual size specific exploitation rates (u) for lower Columbia River white sturgeon fisheries were calculated as the ratio of marks harvested to marks-at-large (Ricker 1975). Tag groups were released each year between April and June. Marks recovered within one year of release were expanded by the fishery sample rate to estimate total mark harvest. Recreational fishery sample rates were estimated by applying the number of fish seen during random fishery sampling (in-sample) to annual harvest estimates (Melcher and Watts 1995). Commercial fishery sample rates were estimated by applying the number of fish randomly sampled at various LCR landing sites and fish buying stations to annual commercial harvest recorded on Washington and Oregon commercial fish receiving tickets. Mark recoveries in recreational fisheries were expanded by a cumulative tag retention rate to correct for tag loss (DeVore et al. 1993). Commercial mark recoveries were corrected for tag loss by expanding observed tag scars by the sample rate. Tag scars were proportioned to all tag groups (tag application year) observed in the fishery.

Mortality was estimated from a catch curve derived from an age frequency distribution of research fishery catches (Ricker 1975). Catches of white sturgeon during 1990-1991 research fisheries were pooled to create a composite length frequency distribution. Corrections for gear vulnerability were made using mark-recapture data collected from 1983-1991 research fisheries employing nets with similar specifications. Vulnerability correction factors were estimated using the ratio of recaptures to marks-at-large by 10 cm FL intervals (Hamley 1975; Lagler 1978; Beamesderfer and Rieman 1988). Only recaptures within three months of marking were used to reduce growth related bias. The combined length frequency distribution was corrected by dividing the observed frequency in each size class by the relative vulnerability (Beamesderfer and Rieman 1988). The adjusted length frequency was converted to age frequency using pooled age-at-length data collected during 1987-1992.

Instantaneous total mortality rate (Z) estimates were made from the slopes of the descending limb of the log transformed catch curve (Ricker 1975). Estimates correspond to age 12-17 fish, representing age classes recruited to the recreational fishery during 1986-1991 (the period exploitation was measured), and age 23-29 fish, representing age classes that had escaped exploitation. Total mortality of age 18-22 fish was not calculated because these age classes were subject to most of their exploitation prior to 1986.

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Abundance.- The Chapman (1951) modification of the Petersen mark-recapture model for closed populations was used to estimate annual abundance of harvestable size white sturgeon each year from 1986-1993. Fish were marked during April-June research fisheries and recaptured by sampling July-March consumptive fisheries. Confidence limits (95%) were calculated assuming recaptures approximated a Poisson distribution (Ricker 1975). Annual abundance estimates were made for sturgeon at age 10 and age 25 using age-at-length data. Population density was estimated from abundance and surface area estimates for the LCR (Parsley and Beckman 1994).

The effect of applying a closed population estimator to an open population was evaluated by simulating monthly changes in population parameters when migration was varied over a nine month period. Immigration and emigration values of 10% and 30% of initial in-river abundance (at the start of the recovery period) were used. The number marked (M), number captured (C), and recaptures (R) were from 1990 data and held constant for all migration scenarios. Initial abundance, proportion marked, sample rate, and migration timing were varied. Sensitivity of the estimator to all migration occurring in the first three months of the nine month recapture period was compared to scenarios where the migration was spread out throughout the recapture period. We considered the estimator satisfactory for scenarios resulting in estimates between initial abundance and initial abundance plus immigration.

Productivity.- The production potential of the population was described using relationships between exploitation and yield (kg) per recruit. An age-structured population simulation model, MOCPOP 2.0 (Beamesderfer 1991), was used to calculate yield and reproductive potential for a range of exploitation rates on 82-166 cm FL fish assuming constant recruitment to age 1.

Results

Age, Growth, and Condition- Ages were assigned to 783 fish ranging in age from 1 to 65 years and in size from 17 to 262 cm FL. The relationship that best described the length-at-age data was the following VBGF: $L_t=276.3(1-e^{(-0.0346(t-1.125))})$. Recoveries of 81 OTC-injected fish indicated that one annulus was deposited each year and that techniques used to age LCR white sturgeon were valid. The average net error for all OTC samples was +0.0 years and for individual interpreters ranged from -2 years for 2 winters-at-large to +4 years for 1 winter-at-large. The largest single error was one reading which was off by +2 years. The correspondence ratio for all samples was 86%, with a range from 75% for 5 winters-at-large to 89% for 1 winter-at-large. Individual interpreters had correspondence ratios ranging from 76% to 94%. The most experienced interpreters had higher correspondence ratios than those with less experience.

Paired length and weight measurements were obtained from 5,222 LCR white sturgeon with lengths ranging from 54 to 263 cm FL and weights from 0.7 to 187.8 kg. Relative weight for LCR white sturgeon averaged 112%.

Sex and Maturity- The sex ratio for the 110-166 cm FL size class was 44.7% female and 55.3% male (n=5,729). Fish >166 cm FL had a sex ratio of 46.5% female and 53.5% male (n=71). Fork lengths in the fecundity/FL relationship ranged from 115 to 215 cm while estimated fecundities ranged from 98,200 to 699,000 eggs (n=38, r^2=0.59).

There were 1,271 ovaries examined for stage of maturity with 92% categorized as pre-vitellogenic, 5% as early vitellogenic, 1% as late vitellogenic, 2% as ripe or spent, and <1% classified as unknown. Early and late vitellogenic females were collected throughout the year. This is consistent with a maturation cycle longer than one year (Chapman 1989; Doroshov et al. 1991; North et al. 1993). Mature females were present prior to and during the spawning time frame and rare during other time frames. Mature females comprised 54% of vitellogenic ovary samples collected in February through

June, but only 2% of August through November samples. The lack of maturation data for the months of January, April, July, and December reflect the absence of commercial fisheries or broodstock monitoring activities during these months.

The median length at first maturity was 160 cm FL (μ) with 95% of the females maturing between 124 and 196 cm FL ($\pm 2\sigma$) (Welsh and Beamesderfer 1993). These lengths correspond to ages 24, 16, and 35 years, respectively. The maximum proportion of maturing females was 0.50, corresponding to lengths ≥ 230 cm FL.

Exploitation and Mortality- Estimated exploitation rates (u) in Columbia River fisheries were highest (0.37-0.38) in the 110-138 cm FL interval where fish were vulnerable to both recreational and commercial fisheries. The geometric mean of estimated exploitation of cohorts used in the catch curve analysis was 0.29 (range 0.19-0.38).

Total instantaneous mortality rate (Z) estimates derived from the 1990-1991 catch curve for 94-123 cm FL (ages 12-17) and 152-175 cm FL (ages 23-29) fish were 0.46 and 0.10, respectively (Figure 3). Instantaneous and conditional natural mortality estimates (M and n) were 0.10 and 0.09, respectively for 94-123 cm FL fish. Natural mortality rates (M and n) for 152-175 cm FL fish were 0.10 and 0.09, respectively.
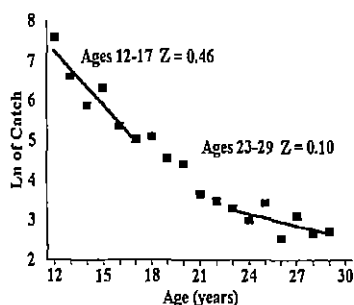
Figure 3. Catch curve for white sturgeon captured during research gill net fisheries on the unimpounded lower Columbia River.

Abundance- There was a decline in abundance of the exploited size classes (92-166 cm FL) between 1986 and 1990, consistent with the high estimated exploitation (Table 1). A significant increase in the abundance of sturgeon beginning in 1991 was primarily due to a regulation change that raised the minimum allowable size limit by 10 cm in 1989. As fish that were spared harvest recruited into the exploitable size classes, abundance increased dramatically. The estimated mean annual abundance of age 10 fish ranged from 124,800 to 189,000 fish; and abundance of age 25 fish ranged between 600 and 1,000 (Table 1). The average density of white sturgeon in the LCR was 2.0 fish per hectare and ranged from 1.3 to 3.1 fish per hectare (Table 1).

Table 1. Abundance of white sturgeon in the Columbia River downstream from Bonneville Dam, 1986-1993, based on mark-recapture estimates.

Year	N	95% C.I.	No./ha	Age 10	Age 25
1986	148,100	110,500-215,300	2.4	188,900	600
1987	146,300	111,700-205,000	2.4	189,000	1,000
1988	111,100	74,900-192,800	1.8	160,700	900
1989	90,000	66,800-132,500	1.5	124,800	600
1990	77,900	59,400-109,400	1.3	161,300	700
1991	87,800	67,400-119,100	1.4	162,800	700
1992	135,300	107,500-193,800	2.2	231,400	600
1993	196,000	157,700-250,200	3.2	157,700	700
Average	124,100		2.0	172,100	700

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Sensitivity analyses indicated that the recapture sampling strategy as well as the magnitude and timing of immigration and emigration influenced the accuracy of the modified Petersen estimator. The estimator was satisfactory (i.e. estimates were within the range of initial abundance and initial abundance + immigration) for low rate of migration scenarios, and for high migration when the migration was spread out throughout the nine month recapture period (Table 4). There was a slight bias, where the estimator overestimated initial abundance + immigration, for high migration scenarios when all the migration occurred in the first three months of the recapture period. The estimator generally predicted the combined population (initial abundance plus immigration) better than initial abundance. Estimates, using all scenarios modeled, were within ±9% of the combined population.

Productivity- Population simulation using constant recruitment predicted maximum sustainable yield (MSY) at 3.0 kg per recruit with an exploitation rate of 0.18 (Figure 5). Estimated sustainable annual yield, based on the number of age 1 recruits, was 1,198,400 kg (19.6 kg/Ha).

Discussion

The LCR supports the greatest number and density of white sturgeon in the three identified production areas (Sacramento/San Joaquin, Columbia, and Fraser basins). Kohlhorst (1980) estimated the abundance of the Sacramento/San Joaquin estuary population (≥ 92 cm FL) to be 40,000 in 1968 and 74,500 in 1979. Abundance increased in the 1980's: 120,000 in 1984, 108,00 in 1985, and 106,000 in 1987, but decreased in the 1990's: 37,000 in 1990, 23,000 in 1993, and 26,000 in 1994. (Kohlhorst 1996; Personal communication, unpublished data). The 1986-1993 average abundance for the same size classes of LCR white sturgeon was 124,100. Estimated abundance of Columbia Basin populations upstream from Bonneville Dam were also lower than LCR estimates (Cochnauer et al. 1985; Lukens 1985), ranging from 870 fish in the Kootenai system (Apperson and Anders 1990) to 51,400 fish (≥ 54 cm FL) in Bonneville Reservoir (Beamesderfer et al. 1995). Annual harvest and catch rate estimates of Fraser River sturgeon (Semakula and Larkin 1968; Parks 1978) indicate that abundance and density may be substantially lower than for the LCR population. It is not clear if this difference is due to overexploitation or relative production potential. More recently, because of decreasing harvest and catch rates, all retention of sturgeon has been prohibited in the Fraser River.

Mean relative weights are higher for LCR white sturgeon than for any other white sturgeon population reported (Figure 4) (Beamesderfer 1993). White sturgeon from the LCR appear to have better growth than other white sturgeon populations. A comparison of VBGFs from white sturgeon population in the three impoundments upstream from Bonneville Dam on the Columbia River and the LCR population indicated significantly higher growth rates for the LCR population (Figure 4) (Beamesderfer et al. 1995). The difference in growth rates between impounded populations and the LCR population may be greater than predicted based on age validation studies. Results from LCR sturgeon age validation samples indicate that 86% of LCR white sturgeon deposited one distinct annulus for each winter at large, and that over ageing was as likely as under ageing. Rien and Beamesderfer (1994) reported an accuracy rate for white sturgeon from Columbia River impoundments at large for 1-3 years of only 35%, and that under ageing was 5.4 times as likely as over ageing. Under aging samples would result in over estimating growth rates and potential productivity.

Superior growth rate and condition of LCR white sturgeon is probably due to adequate habitat and abundant food resources associated with marine-based prey species. White sturgeon in the LCR have access to the marine environment and make seasonal migrations to feed on eulachon (*Thaleichthys pacificus*), northern anchovy (*Engraulis mordax*), American shad (*Alosa sapidissima*), moribund salmonids (*Oncorhynchus spp.*), amphipods (*Corophium spp.*), and other invertebrates (Bajkov 1951; McCabe et al. 1993). The biomass of prey species is high and well distributed seasonally. Therefore, LCR white sturgeon can subsist on alternative resources in the event of declines in usual prey species.

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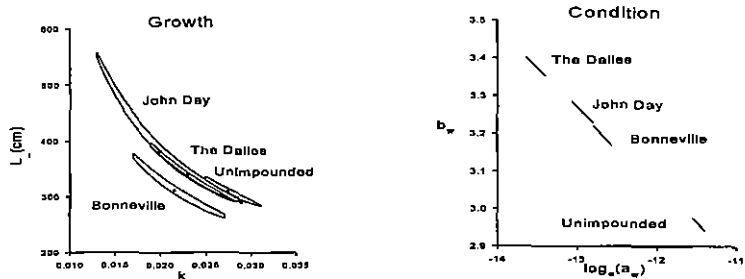
Figure 4. Comparisons of growth (von Bertalanffy growth function) and condition (relative weight) parameters for four Columbia River white sturgeon populations using joint 95% confidence regions. Parameter pairs are considered significantly different if point estimates are not within the confidence region of another area. Reproduced from Beamesderfer et al. (1995).

Population simulation indicates that the LCR population can withstand high exploitation relative to other sturgeon populations. Potential yield for the LCR population at MSY (18% annual exploitation of the 82-166 cm FL population) was 2.9 kg/recruit whereas yield for impounded populations ranged from 0.6-0.8 kg/recruit (MSY exploitation ranged from 5%-15% of the 82-166 cm FL population) if recruitment was assumed constant (Figure 5) (Beamesderfer et al. 1995). Rieman and Beamesderfer (1990) state that, in an unaltered environment, white sturgeon recruitment may be independent of parental stock at high abundance. However, the recovery of the LCR population after protection of broodstock with a maximum size limit demonstrates a relationship between stock size and recruitment. The uncertainty of the degree of dependence of recruitment on parental stock indicates that management strategies regarding sustainable yield should be conservative.

Adequate spawning habitat in the LCR has provided consistent annual recruitment to the young of the year age class while impounded areas may have experienced repeated year class failures (Parsley and Beckman 1994). Parsley and Beckman (1994) demonstrated that hydroelectric development reduced the amount of suitable spawning habitat downstream from Bonneville Dam, although suitable habitat exists in the Bonneville Dam tailrace at lower discharges than in upstream spawning areas (Figure 6). Kohlhorst et al. (1991) and Stevens
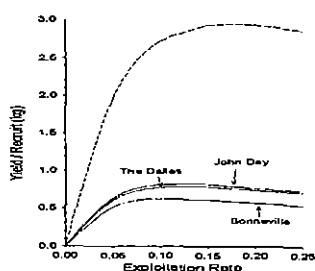
Figure 5. Simulated yield in relation to exploitation rates under an 82-166 cm FL size window for four white sturgeon populations in the lower Columbia River. Reproduced from Beamesderfer et al. (1995).
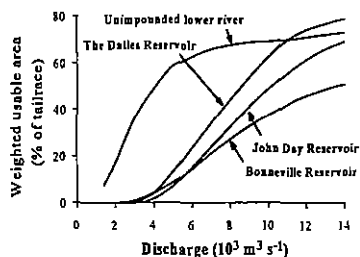
Figure 6. Weighted usable spawning area for white sturgeon at discharges in four lower Columbia River tailraces. Reproduced from Parsley and Beckman (1994)

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and Miller (1970) correlated spawning success with flows in the Sacramento-San Joaquin system. Because of diversions from those rivers and recent droughts, and the recent decline in abundance, it is probable that repeated year class failures occurred in that system too.

A combination of consistently available spawning habitat, adequate rearing habitat, access to the marine environment and associated food sources, and relatively unpolluted water have resulted in a highly productive sturgeon resource in the lower Columbia River. The production of the LCR white sturgeon population combined with conservative management strategies has allowed fisheries to prosper while the population of sturgeon has grown. The LCR recreational sturgeon fishery is the most popular fishery in the region based on annual number of angler trips and increases in popularity every year. A significant commercial harvest also occurs in conjunction with salmon fisheries. These activities add to the economic base of the region and demonstrate successful cooperative fish management in the Columbia River.

Managers should be aware that while LCR white sturgeon may be able to withstand relatively high harvest rates compared to other white sturgeon populations, there is a danger of overexploitation leading to a decline in productivity. Sturgeon throughout their range have a common history of decline or depletion due to overexploitation and habitat changes (Rochard et al. 1990). Optimal sustainable yields (OSY) can only be achieved for the LCR population with conservative management schemes. Harvest rates above OSY that occurred in the late 1980's and the full effect of hydroelectric development of the Columbia Basin will not become fully evident for 20 or more years as declining recruitment to broodstock reduces production. Despite the great production potential of this population, history has taught us that this resource is vulnerable to collapse from overexploitation. Therefore, our challenge is to manage the resource for long term sustainable exploitation and protect critical habitats needed to maintain high productivity.

Acknowledgments

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IMPLICATIONS OF ECOSYSTEM COLLAPSE ON WHITE STURGEON (*ACIPENSER TRANSMONTANUS*) IN THE KOOTENAI RIVER, IDAHO, MONTANA AND BRITISH COLUMBIA.

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Abstract- The Kootenai River ecosystem in Idaho, Montana and British Columbia, (B.C.) Canada has been severely degraded during the past 50 years. This aquatic ecosystem has changed from culturally eutrophic to oligotrophic due to separation of the river from it's floodplain (channeliziation and diking), impoundment (construction and operation of Libby dam) and pollution abatement. The interaction of these three factors and the resulting trophic effects over a period of decades appear to be responsible for the collapse of the Kootenai River ecosystem, and the measurable symptoms of declining and endangered fish populations. The white sturgeon (*Acipenser transmontanus* Richardson) population in the Kootenai River was listed as endangered on September 6, 1994 (59 FR 45989) under the authority of the Endangered Species Act of 1973, in response to the virtual absence of natural recruitment during the past two decades, and an aging and declining population size. Natural recruitment since 1974 consists of 12 white sturgeon. Since the late 1980's, white sturgeon research focused primarily on augmenting discharge in the post-impoundment Kootenai River in order to restore natural recruitment of white sturgeon. Given the complex nature of ecosystem changes in post-impoundment large river systems, it does not appear prudent to expect increased discharge of nutrient deficient water alone to complete the white sturgeon life cycle in the Kootenai River. This paper takes a holistic view of the Kootenai River ecosystem and the ecological responses to development in the Kootenai River Basin, and discusses implications of ecosystem collapse on white sturgeon life cycle completion failure.

The Kootenai River ecosystem in Idaho, Montana and British Columbia, (B.C.) Canada has been severely degraded during the past 50 years. This ecosystem, like other large river-floodplain ecosystems was historically characterized by seasonal floods that promoted the exchange of nutrients and organisms among a mosaic of habitats, resulting in enhanced biological productivity (Junk et al. 1989; Bayley 1995). The Kootenai River ecosystem has changed from culturally eutrophic to oligotrophic due to separation of the river from it's floodplain (channeliziation and diking), impoundment (construction and operation of Libby dam) and pollution abatement (Northcote, 1973; Daley et al., 1981). The interaction of these three factors and the resulting trophic effects over a period of decades appear to be responsible for the collapse of the Kootenai River ecosystem, and the measurable symptoms of declining and endangered fish populations.

The white sturgeon (*Acipenser transmontanus* Richardson) population in the Kootenai River was listed as endangered on September 6, 1994 (59 FR 45989) under the authority of the Endangered Species Act of 1973, in response to the virtual absence of natural recruitment during the past two decades, and an aging and declining population size. The last substantial year-class was produced naturally in 1974, the year that Libby Dam began operation resulting in drastically altered hydrographs, thermographs, and downstream nutrient loading rates. Fisheries research since 1991 has confirmed natural spawning in four of the past five years, however, natural recruitment has been virtually absent. Natural recruitment since 1974 consists of 12 white sturgeon.

Since the late 1980's, white sturgeon research focused primarily on augmenting discharge in the post-impoundment Kootenai River in order to restore natural recruitment of white sturgeon. Based on results of natural recruitment evaluation since 1990 with adaptive river management, discharge augmentation alone has not restored wild white sturgeon recruitment in the Kootenai River. Given the complex nature of ecosystem changes in post-impoundment large river systems, it does not appear prudent to expect increased discharge of nutrient deficient water alone to complete the white sturgeon life cycle in the Kootenai River. This paper takes a holistic view of the Kootenai River ecosystem and the ecological responses to development in the Kootenai River Basin, and discusses implications of ecosystem collapse on white sturgeon life cycle completion failure.

Study Area

From headwaters in southeastern B.C., the Kootenai River flows south into northwest Montana where it is impounded by Libby Dam near the town of Libby, forming Lake Koocanusa (Figure 1). Downstream from Libby Dam, the Kootenai River flows westerly into the northeast corner of Idaho, turns north, enters B.C. and Kootenay Lake. The Kootenay River then flows out the West Arm of Kootenay Lake, joining the Columbia River at Castlegar, B.C. Kootenai Falls, Montana and Bonnington Falls, B.C. act as upstream and downstream migration barriers, are believed to have isolated white sturgeon to a 270 km reach of the Kootenai system in Montana, Idaho, and B.C.

The Kootenai River consists of three habitat types or reaches: canyon, braided channel, and meandering. Immediately downstream from Libby Dam, the river flows through a narrow steep-sided canyon into Idaho. This canyon reach is characterized by swift water and substrates of gravel and larger particle size. The braided channel reach is located from Bonners Ferry, Idaho upstream 10 km and is characterized by reduced gradient, and shallow braided gravel channels. The meandering reach of the Kootenai River is located from Bonners Ferry downstream to Kootenay Lake (Figure 1).

Pre-impoundment

The pre-impoundment Kootenai River (before Libby Dam completion in 1974) hydrograph was characterized by average spring discharge peaks of approximately 1,600 cubic meters per second (m^3/s; 58,000 cfs) (Figure 2). The pre-impoundment Kootenai River was also characterized by a 4 to 6 km wide floodplain in the furthest downstream 128 km of the river. Diking of the furthest downstream 128 km of the Kootenai River (from 1920's to 1950's) in Idaho eliminated approximately 50,000 acres of natural floodplain from Bonners Ferry, Idaho to the International Border. Estimates of floodplain loss due to diking in B.C. may be equal or greater. Natural year classes of white sturgeon were frequently produced in the Kootenai River before 1974, which provided viable commercial and recreational fisheries.

Post-impoundment

The post-impoundment Kootenai River can be characterized as a collapsed aquatic ecosystem following diking (channelizing), impoundment, and denutrification during the past 70 years. The effects of ecosystem collapse on early life stages and lack of juvenile recruitment remain unknown. The post-impoundment Kootenai River (after Libby Dam completion in 1974) is characterized by peak spring discharges up to 67% less than pre-impoundment values (Figure 2). Libby Dam (and the impounded Lake Koocanusa) have acted as a nutrient sink effectively reducing downstream transport of phosphorous and nitrogen by 63 and 25 % (Woods, 1982), with sediment trapping efficiencies exceeding 95 % (Snyder and Minshall, 1995).

In addition to the hydrograph alteration, natural water temperature regimes have also changed in the river since construction of the dam in the early 1970's and selective withdrawal system completion in 1977. Between 1967 and 1972 average water temperatures in the Kootenai River were at or slightly above 0° C from December through February, and peaked in late July and early August and declined rapidly in the fall. Water temperatures were normally near 0° C (32° F) about 3 months of the year, and above 10° C (50° F) for about 4 months with peak temperatures reaching 20° C (68° F). Summer temperatures in the Kootenai River downstream from Libby Dam between 1972 and 1977 were also low due to hypolimnetic withdrawal (Snyder and Minshall, 1994).
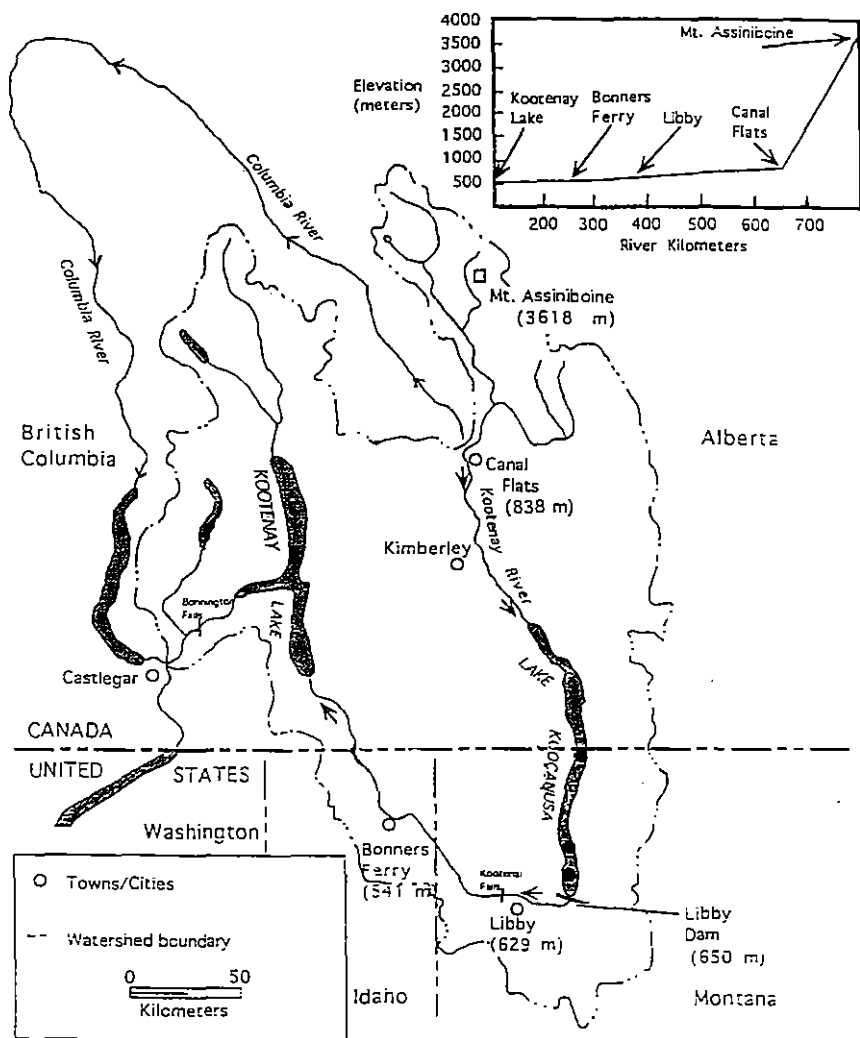
Figure 1. Kootenai River drainage.
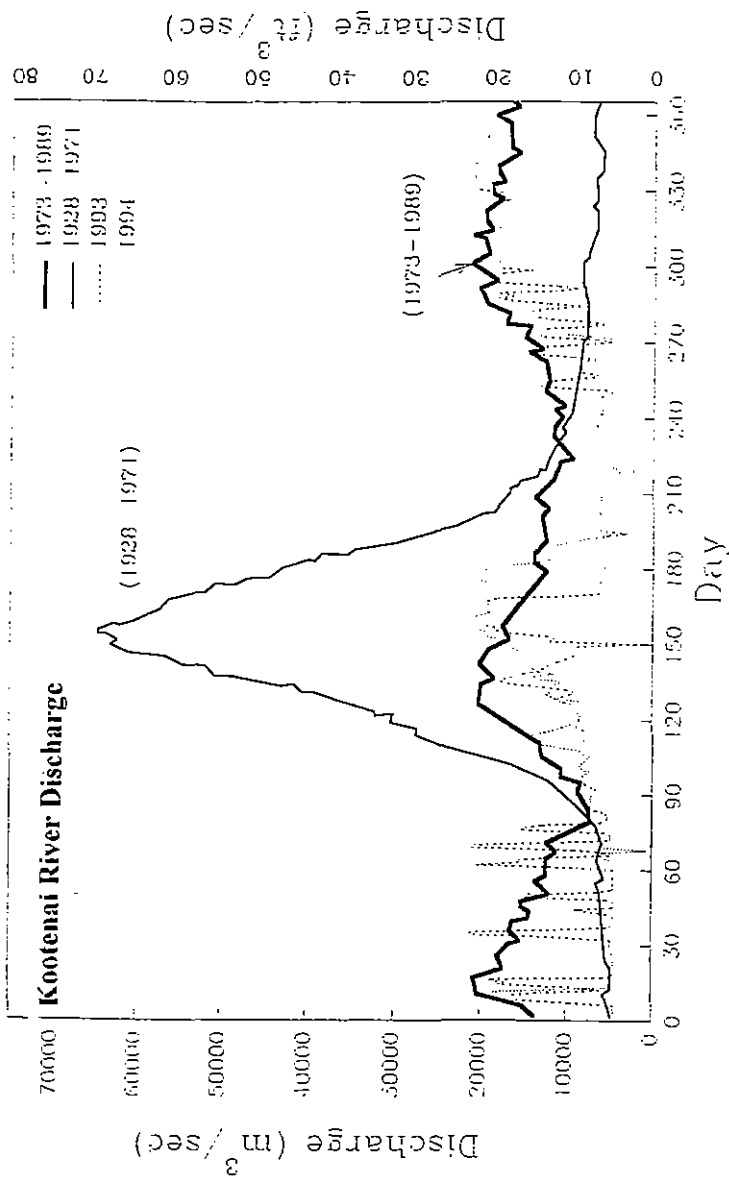
Figure 2. Discharge records from pre- (1928–1971) and post– (1973–1989; 1993–'94) Libby Dam.

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Biological Responses to Kootenai River Basin Development

Depressed primary productivity (ultraoligotrophy), incubation and rearing habitat degradation, fish species composition changes and resulting predation, and declining and endangered fish population status are all biological responses to Kootenai River Basin development. In this paper we will discuss post-development effects on nutrients and primary productivity and fish community dynamics in the Kootenai River system, specifically in the Kootenai River downstream from Libby Dam, and in Kootenay Lake, B.C. (Figure 1).

Kootenai River nutrients/primary productivity

During the past 30 years, the Kootenai River system has regressed from having an excess of nutrients to a system that has become nutrient depraved (Northcote 1973 and Daley et al., 1981). In pre-impoundment years, water quality studies indicated high concentrations of total phosphorus, orthophosphorus, and total nitrogen in the Kootenai River, which were attributed to industrial point-source discharges in the Canadian part of the drainage basin (Bonde and Bush, 1975). During the 1950's, fisherman reported a decline in the water quality of the river characterized by increased algal growth and sedimentation. These effects were attributed to fertilizer plant and mining operations in the basin (MDFWP and USACE, 1983).

As a result of Canadian pollution control measures in the basin and the impoundment of Lake Koocanusa, nutrient concentrations in the river downstream from Libby Dam have declined. Dissolved orthophosphate concentrations averaged 0.383 mg/l in 1970 compared to 0.039 mg/l in 1979. In 1990, Hamilton, et al. reported total and soluble reactive phosphorous concentrations below 0.05 mg/l between 1976 and 1989, and nitrogen concentrations below 0.50 mg/l between 1974 and 1988 in the river. In 1994, Snyder and Minshall reported total phosphorus concentrations ranging from less than 0.005 mg/l to 0.02 mg/l and orthophosphate concentrations from less than 0.005 mg/l to 0.013 mg/l in the Kootenai River downstream from Libby Dam. Wetzel (1983) characterized an oligotrophic system as containing ≤ 0.05 mg/l total phosphorus. Total phosphorous concentrations in the Kootenai River are much lower than this value, characterizing it as deficient or hyperoligotrophic. Inorganic nitrogen ($NO_3 + NO_2$ and NH_4) values ranged from less than 0.01 mg/l and 0.14 mg/l, while total Kjeldahl nitrogen (TKN) ranged from less than 0.1 mg/l to 0.5 mg/l (Snyder and Minshall, 1994). These authors also determined that in the Idaho three river reaches they studied, phosphorus was the nutrient limiting algal growth, and that nitrogen was potentially co-limiting. Historical United States Geological Survey (USGS) nutrient monitoring along with samples collected by Snyder and Minshall in May, July, August, and October 1994 also concur with the 1993 results (Snyder and Minshall, 1994).

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Kootenai River fish communities

A drastic shift in fish species composition downstream from Libby Dam (favoring omnivorous species) has also accompanied impoundment of the Kootenai River. Paragamian (1994) reported a drastic decline in insectivorous fish density from 70 kg/ha to 12 kg/ha; a reduction in growth rates and biomass of mountain whitefish (*Prosopium williamsoni*) was also reported. Mountain whitefish abundance dropped from 480 to 117/hectare following impoundment. The last substantial natural year class of white sturgeon in the Kootenai River was produced in 1974 (Apperson and Anders, 1991). Kokanee salmon (*Oncorhynchus nerka*) runs (South Arm Kootenay Lake stock), numbering thousands of fish as recently as the early 1980's (Partridge, 1983), have declined to less than 85 fish in up to 8 historic spawning streams combined (Anders, 1994; Anders 1993). Catch rates of rainbow trout (*Oncorhynchus mykiss*), and standing stock estimates and growth rates of mountain whitefish in the Kootenai River have declined since the early 1980's (Paragamian, 1994). The burbot (*Lota lota*) population in the Kootenai River has also declined during recent decades, as indicated by an ongoing burbot population study in which 8 burbot were captured during 887 hours of sampling (Paragamian, 1994). Total zooplankton densities in the Kootenai River (mean < 0.1/L) are lower than in other rivers in the northwestern United States (Paragamian 1994).

Kootenay Lake nutrients/primary productivity

In 1953 when Cominco Ltd. began operating a large phosphate fertilizer plant on the St. Mary River (a tributary to the Kootenay River) near Kimberley, B.C., kokanee size in the West Arm of Kootenay Lake increased significantly (Ashley and Thompson, 1993). Cominco tripled its fertilizer production by late 1964, with peak annual losses of phosphate exceeding 8,000 metric tons in the mid to late 1960's (Ashley and Thompson, 1993). Diking of the Kootenai River, Libby Dam operation, and the reduction and eventual closure of Cominco's fertilizer plant on the St. Mary River in 1972, have resulted in reduced Kootenay Lake phosphorus loading to below historical levels. This reduction in nutrient loading has been followed by declines in phytoplankton and zooplankton biomass and kokanee numbers.

In 1992 and 1993, the soluble reactive phosphorus concentration (SRP) was below low level detection limits (<1 µg/l) on most occasions in Kootenay Lake (Ashley and Thompson, 1993, 1994). Inorgainc phosphorus is readily used by plankton and bacteria, therefore the low SRP level exhibited, characterizes a nutrient limited lake (Wetzel, 1983). A decrease in potential primary production can also be inferred as a result of the low SRP concentrations seen in the lake (Jones and Bachman, 1974). Total phosphorus concentrations in the North Arm of Kootenay Lake ranged from 5-10 µg/l in 1992 and 1993, which indicates an oligotrophic to mesotrophic range (Wetzel, 1983). Daley et. al (1981) estimated that phosphorus delivery rates to Kootenay Lake during the spring and summer growing season have been reduced by about 50% because of the presence of Libby Dam and its reservoir (Lake Koocanusa). Throughout these two years, dissolved

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inorganic nitrogen and Kjeldahl nitrogen concentrations remained in the oligotrophic range (<200 µg/l, Wetzel, 1983).

The ratio of inorganic nitrogen to soluble reactive phosphorus (N:P) can be used to determine the relative potential for phytoplankton growth. Generally, nitrogen becomes limiting if the ratio is below 10:1 and phosphorus is limiting at a ratio above 10:1. The N:P ratio of the nutrient loads to Kootenay Lake has declined drastically (approximately 95%) since 1949. The ratio declined from an estimated 14.0 in 1949 to about 0.8 in 1966-1969 and then increased from 1972 to 1977 (Daley et. al., 1981). This fluctuation in nutrient ratio corresponds temporally with the operations of the Cominco fertilizer plant in Kimberley, B.C.

The Kootenai River supplied 75% and 55 % of the measured total phosphorus input to Kootenay Lake in 1976 and 1977, respectively (Daley et. al., 1981). The limnology of the Kootenai River has a profound effect on the aquatic ecosystem of Kootenay Lake, as seen by the relation between nutrient fluctuations in the lake and industrial operations along the Kootenai River system.

Kootenay Lake fish community

Fish species in Kootenay Lake that are important in the recreational fishery include: rainbow trout, kokanee, dolly varden (*Salvelinus malma*), mountain whitefish and burbot (Northcote 1973). Before 1960, the main fishery of the West Arm of the lake focused on small rainbow trout, but by the mid-1960's, whitefish, burbot and kokanee fisheries developed. Shortly after, the burbot and whitefish fishery declined, but the kokanee fishery persisted. By the early 1970's, kokanee catches in the West Arm increased to over 50,000 fish per year (Daley et al., 1981). There was also a steady increase in the size of West Arm kokanee from the 1940's and 1950's to the 1960's and 1970's due to the greater availability of food, mainly mysids (Daley et al., 1981). Northcote (1973) showed through stomach analysis that the large increases in the size of West Arm kokanee were the result of the introduction of mysids to the lake. Since 1970, there has been an increase of the trophy rainbow fishery into the south arm an a rapid expansion of the kokanee fishery in the north arm. Whitefish and burbot are caught primarily in the West Arm. The West Arm of the lake is rapidly flushed with insects and plankton that are transported from the main lake, over the shallow West Arm sill. This has proven to be a good food source for fish inhabiting this portion of the lake, mainly kokanee.

Implications of Ecosystem Collapse on White Sturgeon Life Cycle Completion Failure

Research on Kootenai River white sturgeon early life characteristics from 1990 through 1995 documented production of wild white sturgeon eggs (n = 453), however, none were estimated to be more than 60 hours old. White sturgeon egg incubation ranges from 13.8 to 8.3 days in water from 10 to 14° C. During these same five years one wild white

sturgeon larva (1 day post-hatch recovered from a northern squawfish *(Ptychocheilus oregonensis)* stomach (Anders, 1995) and no age 0 white sturgeon were collected from the Kootenai River.

Based on these data, in the context of major ecosystem changes in the post-impoundment Kootenai River, it is apparent that factors in addition to river discharge are responsible for white sturgeon life cycle completion failure. Early life mortality factors in the post-impoundment Kootenai River responsible for white sturgeon life cycle completion failure may be numerous.

Early life mortality factors

Minimal natural white sturgeon recruitment since 1974, and the preceding general population decline since the mid-1960's are symptoms of ecological deterioration of the post-impoundment Kootenai River system. Repeated recruitment failure since 1974 appears to be a more imminent threat to this population than the perceived inability of this population to reproduce naturally. Therefore, research should be focused on early life mortality factors responsible for recruitment failure (Figure 3). More importantly, white sturgeon research and recovery activities should be immediate and remedial to begin protecting this population's genetic variability and restoring a healthy age-class structure dominated by sexually immature individuals.

Eggs

White sturgeon egg mortality in the Kootenai River may be caused by: incubation habitat loss or degradation, egg suffocation, predation, and contaminants, or post-impoundment conditions resulting in spawning over presently marginal incubation habitat (Figure 3). All but 21 naturally spawned viable white sturgeon eggs collected during the past five years were collected from habitat that currently appears to be suboptimal for incubation. Historical evidence suggests that this reach of river was characterized by the present substrate characteristics. These areas lack interstitial space, and are depositional areas for fine sand, silt and sediments. Conte et al. (1988) reported that an adhesive jelly layer surrounds white sturgeon eggs throughout early development. They also reported that "In natural spawning the adhesiveness of the jelly is important for anchoring the egg to the substrate at the vegetal pole. Such an attachment would orient the micropyle upward before fertilization". Contact with freshwater causes the jelly layer to hydrate within five minutes and the egg becomes adhesive. Most wild white sturgeon eggs collected from the Kootenai River since 1991 (up to 53 hr old when collected) were partially or totally covered with fine sand grains. The fact that no stageable eggs collected from the Kootenai River since 1991 had developed longer than approximately 53 hours in the Shorty's Island area, (characterized by fine substrates), suggests that egg suffocation and predation may be substantial early life mortality factors.

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Figure 3. Potential early life mortality factors for white sturgeon in the Kootenai River to be considered in re-establishment of natural recruitment.

NATURAL SPAWNING — MORTALITY FACTORS

Fertilized eggs → Egg suffocation / Predation / Contamination

Naturally produced white sturgeon larvae

Larval starvation / Food limitation / Rearing habitat degradation

Naturally produced YOY white sturgeon

MORTALITY

YEAR-CLASS FAILURE

Naturally produced yearling white sturgeon

Over-wintering energy deficit

Naturally produced age I and older white sturgeon — YEAR-CLASS PRODUCTION

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Larvae

With the exception of one larval white sturgeon collected from the stomach of a northern squawfish in 1994 in the Shorty's Island area (Anders, 1995) no naturally produced white sturgeon larvae have been collected from the Kootenai River from 1991 through 1995 despite using gear proven to work in other river systems (Palmer et al., 1988; Parsley et al., 1993; Anders and Beckman, 1993; McCabe and Tracy, 1993). Assuming that naturally spawned eggs are able to hatch in the Kootenai River, larval mortality may be caused by: post-impoundment rearing habitat loss or degradation, predation, food limitation, and larval starvation (Figure 3).

Brannon et al. (1985) reported that "Substrate composition in a river may influence both the emergence and settling response of white sturgeon larvae and could affect whether they remain in an area once they become bottom oriented". These authors also reported that "Upon hatching, larvae enter the water column and are subject to the influences of current. Larvae then seek the substrate for places that provide cover. Larvae remained in the substrate until yolk is absorbed and feeding initiated. Larvae were noted to enter just about every conceivable space where they could hide their head. Beneath rocks, gravel interstices, amongst plants, and under detrital material were the places harboring the larvae during the "hiding" phase". Larval white sturgeon were observed in aquaria to lethally burrow into fine sediments (Ernie Brannon, pers. comm.). The fact that larval rearing habitat for white sturgeon in the Kootenai River lacks cover and interstitial space suggests that predation and suboptimal rearing habitat appear as important early life mortality factors.

Young-of-the-year

Food limitation, and first overwintering mortality may contribute to white sturgeon recruitment failure in the Kootenai River. Scott and Crossman (1973) reported that age 0 white sturgeon diets consisted predominantly of *Chironomid* larvae. The amphipod *Corophium* accounted for 98 % of diet items from 149 age 0 white sturgeon (20-267 mm TL) collected from Bonneville and The Dalles pools in the Columbia River from 198 through 1991 (Sprague et al. 1993). Whydowski and Whitney (1979) reported that the stomach of small white sturgeon in California contained primarily mysis shrimp and amphipods. Age 0 lake sturgeon (*Acipenser fulvescens*) were observed in close contact with the substrate, oriented upstream, apparently feeding on drifting benthic organisms (Kempinger, 1996). Kempinger (1996) also reported that species of Baetidae nymphs and Dipteran larvae were the two principle organisms consumed by lake sturgeons during their first summer of life.

Energy requirements and food availability necessary to ensure first overwinter survival of young-of-the-year (YOY) white sturgeon in the Kootenai River are unknown due to the lack of naturally produced YOY. However, low density and diversity of invertebrate food items suggests that food limitation and potential deficits in the first over-wintering energy

budget for YOY white sturgeon may contribute to natural recruitment failure in the Kootenai River.

Summary

Current conditions of the Kootenai River ecosystem are the results of channelization, denturification and hydropower development and operation. The coincidence of adequate discharge, spawning habitat substrates and velocities, water temperatures, and photoperiod conditions has been altered by Libby Dam operation, and may not be restorable in the post-impoundment Kootenai River. Known denutrification of the Kootenai River downstream from Libby Dam may be responsible for food limitation for larval and young-of-the-year white sturgeon. In addition, increased predation pressure resulting from a post-impoundment fish species composition shift favoring omnivorous species may be prohibiting or greatly reducing hatch and early life survival. Finally, changes in bedload movement and known siltation of current spawning areas may also reduce or prohibit natural recruitment through suffocation of demersal, adhesive white sturgeon eggs in the channelized post-impoundment Kootenai River.

Perhaps nothing short of ecosystem centered remedial actions may be needed to complete the natural life cycle of white sturgeon in the Kootenai River. Whether such management actions take the form of altered dam discharges, artificial nutrification, native species re-introduction, predator reduction, or habitat restoration is currently unknown. When entire ecosystems have been altered for decades, as the Kootenai River system has, restoration will require exceptional multi-agency cooperation and commitment well into the next century.

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KOOTENAI RIVER WHITE STURGEON *ACIPENSER TRANSMONTANUS* SPAWNING CHARACTERISTICS AND HABITAT SELECTION POST LIBBY DAM

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Introduction

The Kootenai River white sturgeon *acipenser transmontanus* provided a viable tribal and sport fishery in Idaho for many years until a dam at Libby, Montana was completed in 1972. Data collected during 11 years of white sturgeon investigations in the Idaho portion of the river, 5 years in Montana, and, and British Columbia, Canada, suggested very little recruitment was occurring. Prior to completion of Libby Dam the river had suitable spawning habitat by Columbia River standards (Parsley and Beckman 1994). Examination of the physical and chemical attributes of the river, after construction of Libby Dam, indicated; the river hydrograph was reversed (Figure 1) (high flows in the winter and low flows in the summer), the river was warmer in the winter, and the river was less productive (Snyder and Minshall 1995).

Population studies showed that juvenile white sturgeon were nearly absent from the population. A sample of 185 adult sturgeon examined in Idaho between 1977 and 1980 revealed 79% (144) of these fish were 15-27 years old. Thus, the majority of the of the sample were hatched between the years 1951 and 1965. Hydrographic records indicated these were wet years with better than average runoff. Historic pre-dam flows ranged from 1,416-2,832 m³/s (50,000-70,000 cfs) during the sturgeon spawning period. Peak flows of the Kootenai River after Libby Dam were generally in the range of 250-450 m³/s (8.8-15.9 kcfs) (Apperson and Anders 1991).

Electrophoretic analysis indicated the Kootenai River white sturgeon was a distinct population of white sturgeon (Setter and Brannon 1990). In September of 1994 this population of fish was added to the Federal Registry as an Endangered Species. Since 1991 the Bonneville Power Administration and U.S. Army Corps of Engineers have cooperated in providing flows for sturgeon spawning. Augmented discharge schemes were designed to encourage the white sturgeon to spawn in the Kootenai River, preferably at or above the U.S. Hwy 95 bridge (rkm 245) in Bonners Ferry where gravel and cobble substrates provide increased opportunity for survival of eggs and larval sturgeon. This document summarized the movements, spawning behavior, and habitat use in the Kootenai River during experimental discharge periods for sturgeon spawning.
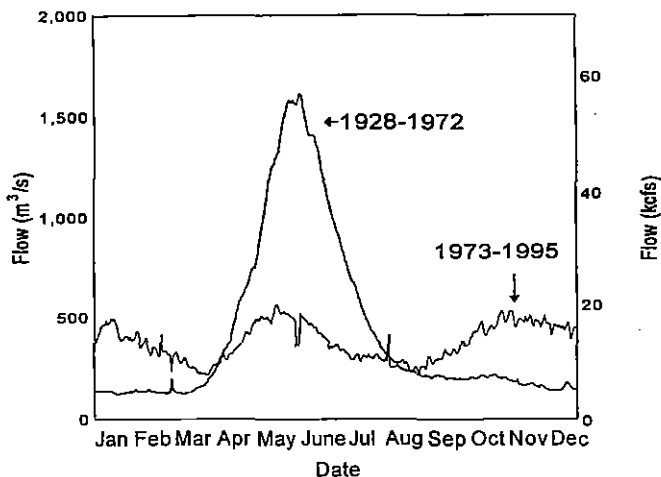
Figure 1 Mean monthly flow patterns in the Kootenai River at Bonners Ferry from 1928-1972 (pre-Libby Dam) and 1973-1995 (post-Libby Dam).

Objective

1. Determine the environmental requirements for adequate spawning and recruitment of white sturgeon.

Study Site

The Kootenai River is in the upper Columbia River drainage. The river originates in British Columbia, flows through Montana and Idaho, and returns to British Columbia. It has a drainage of about 35,490 km² (13,703 mi²).

Methods

Discharge and Temperature

Augmented flow discharge from Libby Dam was provided from 1991 through 1995. Various experimental flow and temperature schemes were provided to simulate natural spring flows and temperatures that occurred prior to construction of Libby Dam. Migration behavior of tagged white sturgeon and a rise in river temperature were the principal means to determine the timing of discharge tests.

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Artificial Substrate Mat Sampling

Artificial substrate mats were used to validate white sturgeon spawning in 1991-1995 (McCabe and Beckman 1990). Mats were set in the river at various locations and times from early May through the end of July. Mats were set in respect to locations and movement of radio and sonic-tagged adult sturgeon that were potential spawners. Mats were pulled and examined for presence of eggs weekly in 1991, every 2-13 days in 1992 and 1993, and daily in 1994 and 1995. Time, depth, temperature and location were recorded at deployment and retrieval for each mat. Bottom and surface velocities were recorded at egg collection sites. Eggs were removed from mats and stored in labeled vials containing formalin or alcohol solution. Embryonic stages of white sturgeon eggs (Beers 1981) were distinguished visually with a dissection microscope. Spawning dates and times ($\pm$4 hours) for all viable eggs were back calculated for nearly all viable white sturgeon eggs using an exponential function involving water temperature and embryonic development as described by Wang et al. (1985) and Beers (1981).

White sturgeon spawning activity in relation to migration of adults, flows, temperature, and gage height

Augmentated flow designs varied in length and intensity between years, but generally lasted from early May through mid-July (Table 1). Water temperature was affected by discharge from Libby Dam. Gage height was dependant upon flows from Libby Dam and the Kootenay Lake level. As with flows, gage height at Bonners Ferry also decreased following the commencement of operation at Libby Dam.

Sturgeon appeared to migrate into the spawning area in the river after flows began increasing during early to mid-May. Males appeared to come and go from the spawning area more frequently than females. Sturgeon tracked into the most suitable spawning habitat appeared to move into these areas as flows were steadily increasing. Most of the females arrived in the spawning area by the end of May and left by mid June. Most males that displayed spawning behavior arrived at the spawning areas by the last week of May, the first one arriving as early as May 1 and the last leaving by June 30. Most females stayed in the spawning area for a total of 1-4 days during May and June and males stayed 4-49 days. Three of the males appeared to spawn during 2 different years with 1-4 years between spawning events. Ten males appeared to spawn during only one year.

A total of 191,180 hours of sampling with artificial substrate mats was expended to verify natural spawning of sturgeon in the Kootenai River between 1991 and 1995 (Table 1). Catch per unit effort (CPUE), a unit of effort was one hour, in less suitable habitat was .00014 eggs/hr. The CPUE in the most suitable spawning habitat was .00017 eggs/hr.

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Table 1. Sample dates, effort, number of eggs collected and depth for artificial substrate mate sampling sites, as well as velocity, temperature and flow at egg collection sites on actual spawning dates in the Kootenai River, Bonners Ferry, Idaho, 1991-1995.

Year	Dates Sampled	Effort (h)	# Eggs Collected	CPUE	Depth (m)	Velocity (m/s)	Temp ©	Flows (m³/s)
1991	5/30-7/12	2,300	13	.0057	6-12	.8-1.0	10.0-16.0	13,197-31,624
1992	ND	21,000	0	0	ND	ND	9.5-18[1]	3,653-24,466[1]
1993	ND	41,587	2	.00005	>2.6	.69	10.4-17.2[1]	19,258-19,306[1]
1994	5/15-7/5	57,630	213	.0037	8-16	.28	8.6-15.1	15,117-20,560
1995	5/1-7/12	78,663	163	.0021	5-11	.17	6.3-15.9	26,640-31,453

[1] Data is from 5/15-7/15 because sample dates are unknown

Eggs were collected between May 15 and July 3 in 1991, 1993, 1994, and 1995 (Figure 2 and Table 1). One spawning event was recorded in 1991, 2 in 1993, 12 in 1994, and 17 in 1995. The earliest documented spawning event occurred on May 14, 1994 and the latest on July 1, 1991. Individual mats contained 1 to 53 eggs on any day. No spawning was known to occur in 1992.

Flows at Bonners Ferry from 1991-1995 exceeded 312 m³/s (11.0 kcfs) from May 7, through June 17. Flows at Bonners Ferry during spawning periods in all years of egg collection, ranged from 374-891 m³/s (13.2-31.4 kcfs). Spawning activity in 1993, 1994, and 1995 occurred after the second peak in discharge began to stabilize above 425 m³/s (15.0 kcfs) and when water temperatures exceeded 10° C (50° F). Spawning in 1991 occurred late in the season as discharge decreased from one steady month of flows exceeding 623 m³/s (22.0 kcfs). Flows on the spawning date were around 368 m³/s (13.0 kcfs) and water temperature was 14.5° C. Spawning in 1993 also occurred lat in the spawning season. Eggs were not collected on mats in 1992 and flows were rarely above 510 m³/s (18.0 kcfs) except for 10 days between May 8 and June 6.

Water temperatures in 1992 were 9.0-15.0° C (48.2-59° F) between April 15 and June 10. Water temperatures during recorded spawning periods in 1991-1995 ranged from 11-14° C (51.8-57.2° F), mat depth ranged from 2.6-16 m (8.5-52.5 ft) and water velocities were from .18-1 m/s (.59-3.3 ft/s) (Table 1). Substrate type at collection sites in 1991 and 1993 were cobble and gravel. In 1994 and 1995, substrate type at egg collection sites consisted of sand.
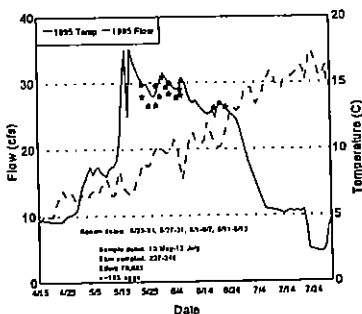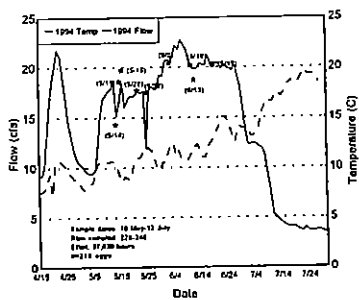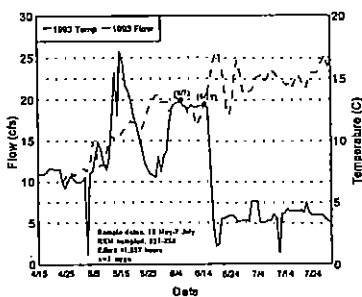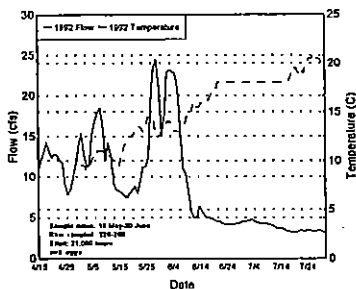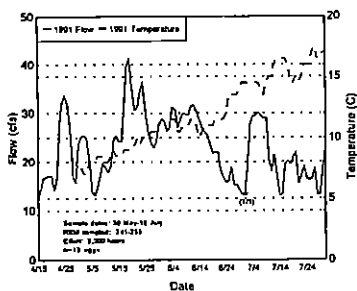
Figure 2. Sturgeon spawning episodes, flow and water temperature, Kootenai River, Bonners Ferry, Idaho, 1991-1995.

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Discussion

Monitoring of the 1991-1995 flows indicated Kootenai River white sturgeon spawned but we are still uncertain as to the survival of eggs and larvae. With the exception of 1991, when the highest flows occurred, companion studies have not documented recruitment (Paragamian et al. 1996). Historic spring time flows before Libby Dam during May to July ranged from about 1,416 m³/s (50 kcfs) to nearly 2,832 m³/s (100 kcfs). Although, it is encouraging that sturgeon responded to and spawned during the test discharges in 1994 (Marcuson et al. 1995) and 1995, a higher discharge is still necessary to replicate the 1974 and 1991 discharge at Bonners Ferry that produced the two most important cohorts since completion of Libby Dam. Thus, the test flows in 1993-1995 were still far below what is believed to be the necessary peak total discharge of about 1,416 m³/s (50 kcfs) for suitable sturgeon spawning, available habitat, and survival of eggs and larvae.

The shape of the spring hydrograph in the Kootenai River may be important to sturgeon spawning. Typically a low elevation runoff of snow and rain raises the hydrograph in April, soon followed by decreasing flows. A seasonal rise in flow is created by high elevation snow melt/rain which is warmer in temperature. This second rise in flow in May stimulates adult sturgeon migration to the spawning areas. Examination of spring through summer hydrographs for 1991 through 1995 showed some similarities in flow attributes in some years (Figure 2). The most recent year since 1974 that provided successful sturgeon reproduction and survival was 1991. Heavy spring rains in May and June caused two peaks in discharge at Bonners Ferry to remain above 651 m³/s (23 kcfs) between May 13 and June 18. The hydrograph was very similar to a natural distribution with a rise in flow with low and high elevation runoff. Although there was a rapid decline in discharge beginning June 19 it did not drop below about 368 m³/s (13 kcfs) and was soon followed by a second rapid rise to well over 850 m³/s (30 kcfs).

Most white sturgeon spawning occurred during relatively consistent flows and for a duration of four or five weeks. The most successful years for sampling eggs were 1994 yielding 213 eggs and 12 events, while 163 eggs were collected in 1995 with 17 known events. Each year was an example of relatively stable flows with temperatures of 7.8-12.9° C (46-55 F). The years of lowest egg collections were 1992, with no eggs, and 1993, two eggs and two events. These two years were characterized by two rapid increases in flow and two precipitation decreases. Although temperatures were adequate the duration of suitable spawning flows for sturgeon were too brief.

White sturgeon spawning events in suitable habitats occurred in 1991 and 1993 late in the spawning season. Suitable habitat is thought to occur primarily upstream from rkm 244. These events were also associated with warmer temperatures of 13.1-14.5° C (56-58° F). However, it is not known if sturgeon spawned downstream of this location in 1991 because all egg sampling was carried out upstream of rkm 245.

Still remaining are questions as to the suitability of the location of spawning in 1994 and 1995, the survival of eggs and larvae, and the affect of handling stress caused by the angling of adults for brood fish. A companion study designed to determine a technique to document the abundance of larval or young of the year sturgeon failed to produce evidence of survival (Paragamian et al. 1996). Numerous types of active and passive gears were used in all habitats.

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Kootenai River white sturgeon spawning during 1994 and 1995 took place in habitat thought to be unsuitable by Columbia River standards. Parsley and Beckman (1994) created microhabitat criteria curves depicting the suitability of temperature, water depth, mean column velocity, and substrate. Clean cobble, boulder, and bedrock was the most suitable substrate, whereas Kootenai River white sturgeon spawned over sand in 1994 and 1995. The most suitable mean column velocity ranged from 1.5 to 2.3 m/s (4.9 to 7.5 ft/s) in the Columbia River but Kootenai River white sturgeon spawned in velocities from 0.17 to 1.0 m/s (0.56 to 3.28 ft/s) (Appendix A). Kootenai River white sturgeon spawned at temperatures ranging from 7.5 to 14.5° C (45.5 to 58° F) from 1991 through 1995 while the majority of spawning in the Columbia River took place at warmer temperatures of 12 to 17° C (53.6 to 62.6° F). Water depths of at least 4 m (13 ft) are the only similarities in egg collection sites, but water depth may be the least important variable. It is not known if the disparity in habitat for spawning by Kootenai River white sturgeon and their counterparts in the Columbia River are due to evolutionary traits for survival or that sturgeon in the Kootenai River spawned in less suitable habitat because they are compelled to.

Our goal is to stimulate Kootenai River white sturgeon migration, spawning, and recruitment in order to recreate a self sustaining white sturgeon population. Recent test discharges appear to have stimulated spawning. But because the fate of eggs and larvae remain unknown we believe augmented discharge from Libby Dam should be 708 m³/s (25 kcfs) to allow spawning sturgeon to move into the most suitable habitat. An equal amount of naturally occurring local inflow would provide 1,416 m³/s (50 kcfs) at Bonners Ferry. This magnitude of discharge would replicate 1991 flows (Figure 2) that produced the last notable cohort of sturgeon. Much of the decision to initiate augmented flows to stimulate sturgeon spawning in 1996 will be based on the migration behavior of sturgeon and rise in river temperature to 10° C (53.6° F).

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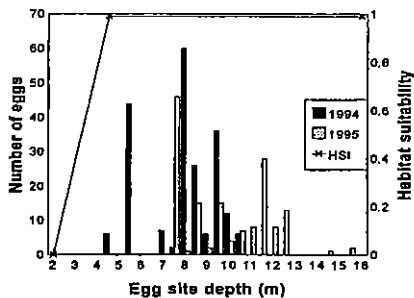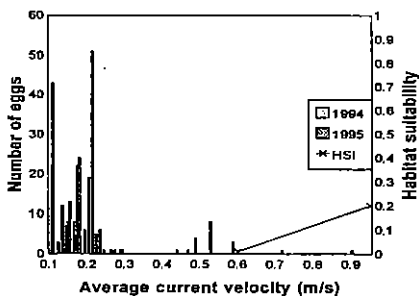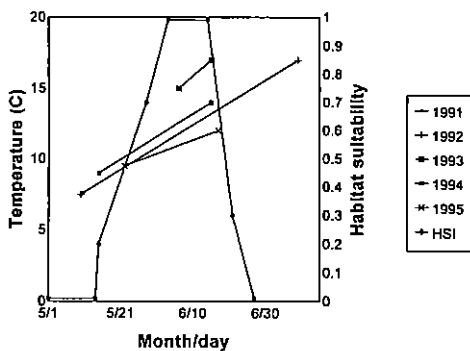
Appendix A. Habitat suitability curves (Parsley and Beckman 1994) and habitats utilized by white sturgeon in the Kootenal River, Idaho. 1991-1995. Top figure is temperature, middle figure is average current velocity and bottom figure is depth.

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CONSERVATION AQUACULTURE OF ENDANGERED WHITE STURGEON

(*Acipenser transmontanus*) FROM THE KOOTENAI RIVER, IDAHO.

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Abstract.- The white sturgeon (*Acipenser transmontanus* Richardson) population in the Kootenai River was listed as endangered by the U.S. Fish and Wildlife Service on September 6, 1994 (59 FR 45989) under the authority of the Endangered Species Act of 1973. This population was listed due to the virtual absence of recruitment during the past two decades, declining population size, and post-development habitat loss and degradation. The last substantial year-class was produced naturally in 1974, the year prior to operation of Libby Dam. In 1990, the population size was estimated to be 880 individuals (88-274 cm TL; 95% CI 638-1,211), of which > 80% were older than age 21. During the late 1980's, assessment of conservation aquaculture with Kootenai River white sturgeon was proposed. During the early 1990's, conservation aquaculture began in order to maintain the population's genetic variability, reduce the threat of extinction, and counteract the lack of recruitment and decline in the size of the Kootenai River white sturgeon population. In 1991, 1992, 1993, and 1995 progeny from wild Kootenai River white sturgeon broodstock were successfully produced and reared in the Kootenai Hatchery. During these years, 7 females were mated with 14 males, producing 9 families of progeny. To date, 208 hatchery-reared progeny of wild Kootenai River white sturgeon have been stocked in the Kootenai River. Thirteen of 104 age 1 hatchery reared Kootenai River white sturgeon released in 1992 were recaptured during 1995. Following three years in the Kootenai River, these fish grew from 19 to 38 cm (TL). Fifteen of 90 age 2 hatchery reared fish were released in 1994 were also recaptured during 1995. Annual growth of these fish ranged from 0 to 10 cm (TL). Given the current status of the Kootenai River white sturgeon population (lack of recruitment, aging population, unknown and declining effective population size) and the uncertainties of recovery efforts to re-establish natural recruitment (augmented discharge), conservation aquaculture is a prudent and necessary recovery tool to ensure the existence of this endangered white sturgeon population for future generations.

The white sturgeon (*Acipenser transmontanus* Richardson) population in the Kootenai River was listed as endangered on September 6, 1994 (59 FR 45989) under the authority of the Endangered Species Act of 1973. This population was listed due to the virtual absence of recruitment during the past two decades, declining population size, and post-development habitat loss and degradation. The last substantial year-class was produced naturally in 1974, the year prior to operation of Libby Dam. The dam impounds the Kootenai River near Libby, Montana, forming Lake Koocanusa (Figure 1). Construction and operation of Libby Dam has drastically altered the hydrograph, thermograph, and downstream nutrient loading rates in the Kootenai River. Research since 1991 has confirmed natural spawning in four of the past five years; however, natural recruitment has been virtually absent since 1974 (n=16).

The Kootenai River white sturgeon population was estimated to be 4,000-6,000 individuals in 1981 (Graham, 1981). Using tag recovery data from 1979-1981, Partridge (1983) estimated the population to be 1,148 fish (50-224 cm TL; 95% CI 907-1503). In 1990, the population size was estimated to be 880 individuals (88-274 cm TL; 95% CI 638-1,211) (Apperson and Anders, 1991). The 1990 estimate was not statistically different from the previous estimate of 1,148, however, these estimates were not directly comparable since they covered different geographic areas (Giorgi, 1993). Apperson and Anders (1991) reported that natural mortality was 3.74%, sex ratio was approximately 1:1, and that 7% of female and 30% of male white sturgeon in the Kootenai River reproduce annually. Assuming a 3.74% annual mortality rate and the 1990 population estimate of 880 fish, 153 wild white sturgeon died between 1990 and 1995. The number of female spawners was approximately 25 in 1995, assuming 7% of the female population was reproductive. Accordingly, another 101 fish will be lost from the population during the next five years (1996-2000) resulting in only 21 reproductive females by the year 2000. No recent population estimates for this population exist. Updated estimates should be completed by the fall of 1996.

Since the formation of Bonnington Falls on the Kootenay River (west of Kootenay Lake B.C.) approximately 10,000 years ago, white sturgeon in the Kootenai River system are believed to have been landlocked between Kootenai Falls, Montana, and Bonnington Falls B.C. (Figure 1). Genetic analysis (electrophoresis) of white sturgeon populations has revealed that the Kootenai River population has the lowest average heterozygosity (0.54) compared to that of white sturgeon populations in the Columbia River (0.74; Setter and Brannon, 1990; Setter and Brannon, 1992). Subsequently, the Kootenai River white sturgeon population has been reported by the U.S. Fish and Wildlife Service as a genetically separate population (59 FR 45989).

During the late 1980's, assessment of conservation aquaculture was proposed. During the early 1990's, conservation aquaculture began to maintain the population's genetic variability, reduce the threat of extinction, and counteract the lack of recruitment and decline in the size of the Kootenai River white sturgeon population. In 1991, an experimental aquaculture program began to assess Kootenai River water quality, white sturgeon gamete viability and the feasibility of aquaculture as a component of population
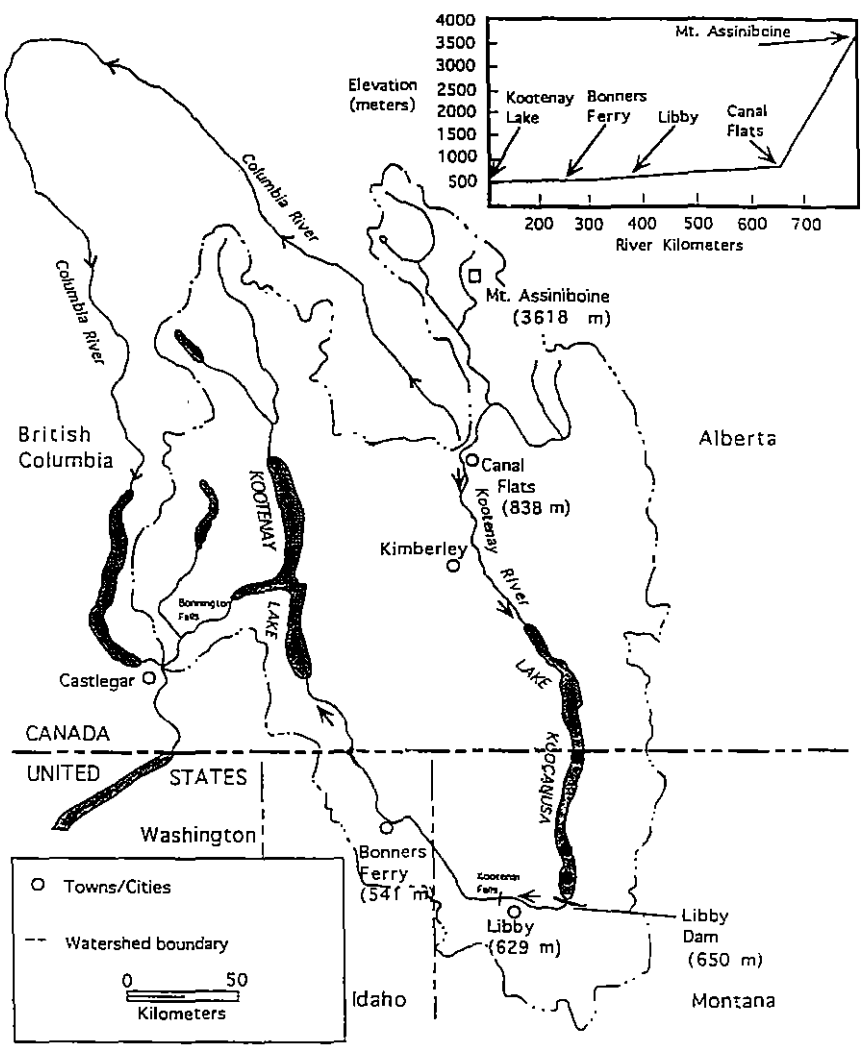
Figure 1. Kootenai River drainage.

recovery. In 1991, 1992, 1993, and 1995 progeny from wild Kootenai River white sturgeon broodstock were successfully produced and reared in the Kootenai Hatchery. In 1993, a breeding plan to preserve the genetic variability of the Kootenai River white sturgeon population was prepared (Kincaid, 1993) to guide operations at the Kootenai Hatchery.

In light of the need to prevent extinction and preserve the remaining genetic variation of the Kootenai River white sturgeon population, the objectives of this paper are to describe benefits and techniques of conservation aquaculture as a component of population recovery.

Study Area

From headwaters in southeastern British Columbia, the Kootenai River flows south into northwest Montana where it is impounded by Libby Dam near the town of Libby, forming Lake Koocanusa (Figure 1). Downstream from Libby Dam, the Kootenai River flows westerly into the northeast corner of Idaho, turns north, enters British Columbia and Kootenay Lake. The Kootenai River then flows out the West Arm of Kootenay Lake, joining the Columbia River at Castlegar, B.C. Kootenai Falls, Montana forms an upstream migration barrier and Bonnington Falls, B.C. blocks downstream migration isolating white sturgeon to within a 270 km reach of the Kootenai River system in Montana, Idaho, and B.C.

The Kootenai River consists of three habitat types or reaches: canyon, braided channel, and meandering. Immediately downstream from Libby Dam, the river flows through a narrow steep-sided canyon into Idaho. This canyon reach is characterized by swift water and substrates of gravel and larger particle size. The braided channel reach is located from Bonners Ferry, Idaho upstream 10 km and is characterized by reduced gradient, and shallow braided gravel channels. The meandering reach of the Kootenai River exists from Bonners Ferry downstream to Kootenay Lake (Figure 1).

Pre-impoundment

Prior to impoundment (before Libby Dam completion in 1974) the Kootenai River hydrograph was characterized by average spring discharge peaks of approximately 1,600 cubic meters per second (m3/s; 58,000 cfs) (Figure 2). The pre-impoundment Kootenai River was also characterized by a 4 to 6 km wide floodplain in the furthest downstream 128 km of the river. Diking of the furthest downstream 128 km of the Kootenai River (from 1920's to 1950's) in Idaho eliminated approximately 50,000 acres of natural floodplain from Bonners Ferry, Idaho to the International Border. Estimates of floodplain area lost to diking in B.C. may be equal or greater. Naturally produced year classes of white sturgeon were frequently documented in the Kootenai River before 1974. The self-sustaining provided a viable commercial and recreational fisheries.

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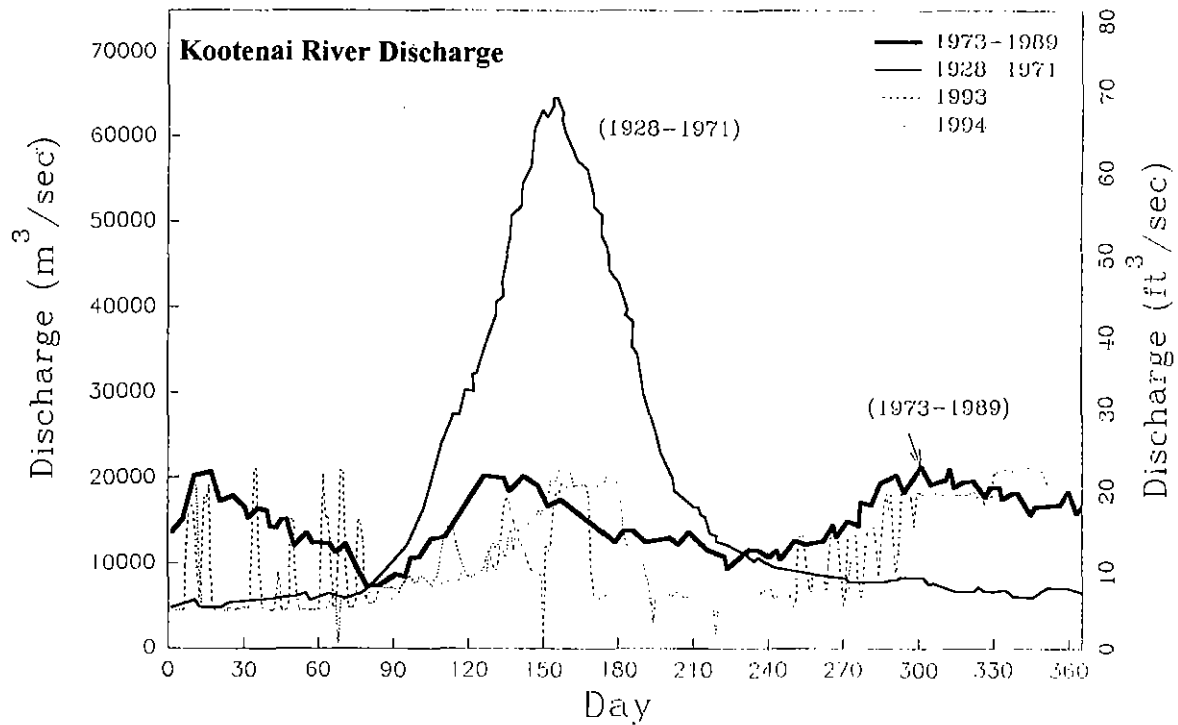
Figure 2. Discharge records from pre– (1928–1971) and post– (1973–1989; 1993–'94) Libby Dam.

Post-impoundment

The Kootenai River can be characterized as a collapsed aquatic ecosystem following diking (channelizing), impoundment, and resulting denutrification. The effects of ecosystem collapse on early life stages and lack of juvenile recruitment remain unknown. After Libby Dam was completed in 1974 peak spring discharges were reduced up to 67% of pre-impoundment values (Figure 2). Libby Dam (and the impounded Lake Koocanusa) have acted as a nutrient sink effectively reducing downstream transport of phosphorous and nitrogen by 63 and 25 % (Woods, 1982), with sediment trapping efficiencies exceeding 95 % (Snyder and Minshall, 1995). A drastic shift in fish species composition downstream from Libby Dam (favoring omnivorous species) has also accompanied impoundment of the Kootenai River (Paragamian, 1994). The last substantial natural year class of white sturgeon in the Kootenai River was produced in 1974 (Apperson and Anders, 1991).

Methods

Broodstock Collection

From April through June 1991, 1992, 1993, and 1995, wild Kootenai River white sturgeon broodstock were collected by angling. Captured fish were placed ventral side up in a hooded vinyl stretcher suspended across the boat gunwales. Fish in the stretcher were periodically provided with fresh river water. Sex and reproductive development of captured fish were determined by visual observation through a 2-3 cm midline incision on the ventral surface of the fish between the 3rd and 4th ventral scutes anterior to the vent. In preparation for examination, this area was treated with a 4% nitrofurazone antibacterial solution. Alison forceps were used to collect a gonadal tissue biopsy to confirm gonad development. All captured males were checked for maturation on the river by inserting a tygon tube (5 mm diameter) into the genital opening and siphoning sperm out with an attached 20 ml plastic syringe. If sperm was collected, or if a gonad inspection determined that male and female white sturgeon were sexually mature, they were transported to the hatchery in an oxygenated tank truck. Non-reproductive males and females were immediately released back into the river.

Reproductive development of males and females was categorized according to criteria reported by Conte et al. (1988). All fish examined were checked for recapture, the removal of a scute, and marked with a PIT tag (Passive Integrated Transponder, Destron Inc. Boise, ID. USA) in the dorsal musculature ventral to the dorsal fin. All fish were measured (FL, TL cm) and weighed, and a pectoral fin ray section was removed for age determination. Each fish was also marked with an individually numbered external dart tag (Floy Tags Inc. Seattle, WA. USA) and by removal of the second anterior left lateral scute. Once all data were collected and sex and reproductive status were determined, fish

were either brought to the hatchery for spawning in an oxygenated tank truck or released immediately back into the river.

Adult female white sturgeon brought into the hatchery were periodically examined for oocyte development and degree of maturation. Examined fish were guided into a hooded vinyl stretcher with the anterior end of the fish covered by the hood. Once the fish was secured in the stretcher it was rolled ventral side up, declined slightly at the anterior end to keep the head submerged. A piece of tygon tubing (3 cm dia.) was then fitted in the mouth of the fish to provide a constant source of oxygenated water over the gills. Using the surgical procedure described above to determine reproductive status of wild broodfish in the field, 10 to 20 eggs were removed and microscopically observed to determine maturation and timing for hormonal induction of ovulation.

Spawning Broodstock

All adult white sturgeon spawned in the Kootenai Hatchery were wild, collected from the Kootenai River by angling one to two months prior to spawning. Adult white sturgeon were spawned in the Kootenai Hatchery during June and July and released back into the Kootenai River after several weeks of post-spawning observation.

The following criteria were used to determine timing of hormone injections to most effectively induce ovulation: 1) appearance, shape, and attresia of eggs; 2) egg diameter through long axis; 3) position of the germinal vesicle (GV); 4) progesterone egg maturation assay producing germinal vesicle breakdown (GVBD), and 5) Oocyte Polarization Index (PI) values (Siple and Aitken, 1992; Conte et al. ,1988; Van Eenaanam et al., 1996).

Females

Once eggs from female broodstock exhibited GVBD (Conte et al., 1988) and a PI value of ≤ 0.12 (Van Eenaanam, 1996), hormone injections to induce ovulation were initiated. Two injections, primary and resolving doses of lutenizing hormone releasing hormone analogue (LHRHa) were administered. The primary dose consisted of 10% of a 0.1 mg/kg body weight injection, followed in 12 hours by a resolving dose of 90% of 0.1 mg/kg body weight LHRHa. Following the resolving dose injection, the female was returned to a 1.0 X 0.67 X 3.0 m holding tank, which was viewed hourly to observe the onset of ovulation. Ovulation was defined by the presence of at least 100 eggs shed into the spawning tank. Depending on water temperature in the spawning tank, egg removal (hand stripping) began 4 to 7 hours after the first signs of ovulation. This 4 to 7 hour delay provided time for eggs to be shed inside the body cavity, allowing subsequent hand-stripping to massage them through the oviduct.

Males

To date, male white sturgeon in the Kootenai Hatchery have not received hormone injections. A 30 ml sperm sample from each male was collected several hours after administering the females LHRHa resolving dose. A portion of sperm from each 30 ml sample was checked microscopically to determine motility duration when water activated. Sperm motility greater than 2 minutes was required for fertilizing eggs. The remainder of unused, motile sperm samples were stored in sealed plastic bags with pure oxygen and refrigerated at 4-5° C.

Egg Removal and Fertilization

During 1991 and 1992 Cesarean surgery was used to remove eggs from the body cavity of ovulating white sturgeon broodstock. In 1993, eggs were collected by Cesarean surgery and hand-stripping. In 1995, eggs were removed only by hand-striping. The Hand-stripping greatly minimizes stress associated with Cesarean surgery and reduces the recovery period of post-spawning adult white sturgeon prior to release back into the wild.

Results

In 1991, 1992, 1993, and 1995 progeny from wild Kootenai River white sturgeon broodstock were successfully produced and reared in the Kootenai Hatchery. During these years 7 females were mated with 14 males producing 9 families of progeny (Table 1). In all years except 1995, sperm from more than one male was combined to fertilize eggs. In 1995, sperm from each male was used to fertilize a separate batch of eggs producing four families, (or two pairs of half-sibling families, each with a shared female parent).

To date, 208 hatchery-reared progeny of wild white sturgeon broodstock have been released into the Kootenai River (Table 2). To date, 208 hatchery-reared progeny of wild Kootenai River white sturgeon have been stocked in the Kootenai River. Thirteen of 104 age 1 hatchery reared Kootenai River white sturgeon released in 1992 were recaptured during 1995. Following three years in the Kootenai River, these fish grew from 19 to 38 cm (TL). Fifteen of 90 age 2 hatchery reared fish were released in 1994 were also recaptured during 1995. Annual growth of these fish ranged from 0 to 10 cm (TL).

Table 1. Crosses of wild Kootenai River white sturgeon in the Kootenai Hatchery, 1991-1995 as part of the conservation breeding program.

Year	Females	Males	# Families Produced
1991	1	2[a]	1
	1	2[a]	1
1992	1	2[a]	1
	1	1	1
1993	1	3[a]	1
1994[b]	0	0	0
1995	1[c]	2	2
	1[c]	2	2
Total	7	14	9

a = Sperm from > 1 male was pooled to fertilize eggs from each female.
b = No fish spawned during 1994.
c = Eggs from each female were fertilized separately with sperm from two different males.

Table 2. Data from twenty-eight hatchery-reared white sturgeon recaptured from the Kootenai River during 1995.

Year class	Number	Release year	Capture year	Percent (#) Recaptured
1990	14	1992	none	0
1991	104	1992	1995	12.5 (13)
1992	90	1994	1995	13.3 (15)
Total	208			13.5% (28)

Discussion

The risks and benefits of conservation aquaculture must be evaluated on a case by case basis. The Kootenai River white sturgeon population is aging (>80% is older than 20 years) and has very limited natural recruitment (n=16) represented since 1974. This population condition will result in a missing generation of spawners in another 15-20 years because the youngest reproductive male and female in this population were estimated to be 16 and 22 years of age. Augmented discharge regimes in the Kootenai River during 1991, 1993, 1994, and 1995 designed to stimulate natural spawning and recruitment have not restored natural recruitment. Juvenile recruitment (natural or artificial) is needed to rebuild the age-class-structure of the Kootenai River white sturgeon population to reduce the threat of extinction and preserve the remaining genetic variation.

To address this problem, a breeding plan to preserve the genetic variability of the Kootenai River white sturgeon population and to begin rebuilding the natural age class structure was developed (Kincaid, 1993). This plan is designed to maximize the number of different adults contributing progeny to the population over time while minimizing contribution of any one sibling group. Under this plan 3 to 6 different females will be spawned annually, and a sufficient number of fish will be released to produce an estimated 10 spawners per family at age 20.

In addition to preserving genetic variability, rebuilding a healthy natural-age-class-structure and preventing extinction, conservation aquaculture of Kootenai River white sturgeon provides other important benefits. Artificial production of early life stages and juvenile white sturgeon using a genetically sound conservation aquaculture program (Kincaid, 1993) will allow identification of limiting factors for wild egg incubation, hatching, and rearing and survival of early life stages in the Kootenai River. Release of hatchery-reared juvenile white sturgeon into the Kootenai River also provides valuable information on growth, habitat use, and feeding ecology.

Given the current status of the Kootenai River white sturgeon population (lack of recruitment, aging population, unknown and declining effective population size), and the uncertainties of recovery efforts to re-establish natural recruitment (augmented discharge), conservation aquaculture is a prudent and necessary recovery tool to ensure the existence of this endangered white sturgeon population for future generations. The Kootenai River white sturgeon conservation aquaculture program, in combination with augmented discharge, (Marotz et al. 1996) is the most reasonable approach to population recovery. Kincaid stated "the notion that these two approaches are incompatible is a misconception. There is no biological reason to prevent simultaneous implementation of both approaches. Indeed, when the advantages and disadvantages of both approaches are considered in light of the current "endangered" status of Kootenai River white sturgeon, simultaneous implementation of both approaches (augmented discharge and conservation stocking) seems to offer the highest probability to protect and preserve the genetic variability of the Kootenai River white sturgeon population".

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NATIONAL DATABASE FOR STURGEON AND PADDLEFISH

INFORMATION, A NEW TOOL FOR FISHERIES MANAGEMENT

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Introduction

A goal of fisheries agencies is to maintain or increase natural stock numbers while preserving the genetic integrity of all stocks under their jurisdiction. The number of declining stocks listed as threatened or endangered under the Endangered Species Act of 1973, as amended: i.e., Kootenai River white sturgeon (Duke, 1994), Pallid sturgeon (Graham et al., 1995) demonstrates that this goal has not been achieved in many managed fisheries throughout the country.

Studies over the past decade have examined relationships between sturgeon populations and human influence on sturgeon habitats, i.e., dam construction, overharvest, environmental pollution, and hatchery supplementation (Binkowski and Doroshov, 1985: Wallus, 1990; Birstein, 1993). Breeding guidelines to manage genetic diversity in fish populations have been developed (Krueger et al., 1981; Kapuscinski and Jacobson, 1987; Ryman and Laikre, 1991). These guidelines and studies also demonstrate the inadequacy of current information on the habitat preferences, life history, genetic structure, and performance characteristics of different broodstocks/populations. Managers need accurate broodstock information (riverine or lake origin, genetic structure, etc.) to identify the broodstocks best suited for different fisheries situation when enhancement or restoration programs are to be undertaken. Complete information on life history, behavior, reproduction, and habitat preferences of different broodstocks is essential if managers are to achieve management goals of stock restoration, stock enhancement, or stock maintenance. Information on stock structure will allow biologists to design programs to protect the natural genetic diversity within the species and assist efforts to restore sturgeon in habitats capable of supporting self sustaining natural populations (Kincaid, 1993).

Broodstock information is especially difficult to obtain for wild populations of long lived species like the sturgeon and paddlefish that have not been cultured (Birstein, 1993; Kincaid, 1993). For many of these broodstocks the information available is not in the published literature, but is in fisheries agency records or in the experience of local culturists and field biologists.

Management plans that include supplementation stocking need to identify the genetic background and

life history characteristics of each native fish and potential broodstock donor sources for each targeted habitat and ecosystem. Complete information (genetic characteristics, life history, and habitat preference) on potential broodstock sources enhances managements ability to effectively manage resources and protect the genetic integrity of each population.

The National Fish Broodstock Registry - Sturgeon and Paddlefish (NFBR-SP) was established in 1996 to catalog habitat preferences, life history, and performance information on managed wild and domestic sturgeon and paddlefish broodstocks. The NFBR-SP is a cooperative project of the Fish and Wildlife Service (FWS), Division of Fish Hatcheries and the National Biological Service (NBS), Research and Development Laboratory. The NFBR-SP contains information on broodstocks of seven Acipenseriformes species found in the United States (Table 1). The term *broodstock* in this manuscript is used to mean an interbreeding population that occurs either as a cultured or free-ranging population i.e., the terms *"broodstock"* and *"stock"* are used interchangeably when referring to natural or free-ranging populations.

Common name	Scientific name
White sturgeon	*Acipenser tansmontanus*
Green sturgeon	*Acipenser medirostris*
Lake sturgeon	*Acipenser fulvescens*
Atlantic sturgeon	*Acipenser oxyrhynchus*
Shortnose sturgeon	*Acipenser brevirostrum*
Shovelnose sturgeon	*Scaphirhynchus platorynchus*
Pallid sturgeon	*Scaphirhynchus albus*
Paddlefish	*Polyodon spathula*

Table 1. Common and scientific names of paddlefish and sturgeon species with broodstock information reported in the National Fish Broodstock Registry. Scientific names taken from: Robins et al., 1991.

Methods

The NFBR-SP was developed in five steps. First, an advisory committee of species experts from throughout the country was assembled to represent federal, state, and private aquacultural interests in identifying information for inclusion in the database. The committee was responsible to develop guidelines for selecting traits to include in the data set, develop a final questionnaire, serve as liaison to broodstock managers and growers, and serve as an editorial board for products developed from the data set. Step two was to review the fish broodstock literature to identify publications, federal aid reports, and agency in-house reports characterizing individual broodstocks. Once identified, copies were obtained to establish a permanent fish broodstock library at the Research and Development Laboratory.

Step three was the national survey of federal and state fisheries agencies and private producers. The surveys consisted of three sections: the Broodstock Section to be completed by broodstock managers, the Culture Performance section to be completed by hatchery managers, and the Field Performance section to be completed by field biologists. Initial questions in each section identified the broodstock, the contact person, and documents describing the broodstock, i.e., publications and reports. Broodstock identification consisted of the species common name, broodstock name used by management, and the strain name. When no strain name was provided, the name of the originating water body or hatchery was assigned. Other questions were designed to identify broodstock origin, current status, broodstock maintenance and breeding procedures, genetic characterizations, and performance characteristics i.e., reproduction, life history, disease tolerance, and stress tolerance. Managers were asked if gametes or fish would be available to other agencies and growers. The contact persons identified for each broodstock provided a mechanism for clarification of specific survey responses and for obtaining new information as it becomes available.

The hatchery section asked for performance information on progeny of the broodstock in cultural situations. Data included information to characterize the production situation; i.e., water quality, feed type, and type of facilities. Requested information included growth rate, survival, feed conversion,

stress tolerance, disease tolerance, and special handling considerations. The field section identified the management situations where the fish were stocked and post-stocking performance characterized. Many questions in the hatchery and field sections were subjective, asking the manager or biologist to rate the performance of the broodstock relative to others stocked in the same or similar situations.

The fourth step was development of the NFBR-SP as an electronic database, including data entry, compilation, and summarization. The NFBR-SP was programmed in the R-Base language. The fifth and last step is ongoing and includes two tasks: periodic updating and maintenance of the database and development of a system to distribute database information to federal and state agencies, private growers, and university user groups.

State/Province	State/Province	Federal	Commercial growers	University	Tribes	Species
Alberta	1					LS
Alabama				1		P
Arkansas	2					P
British Columbia				1		WS
California		1		2		GnS, WS
Florida		2	2	1		GS, AS, SS
Georgia		1				P
Iowa	2					ShS, P
Idaho	1	1			1	WS
Kansas	2					P
Kentucky				1		P
Louisiana	1	3				P
Massachusetts		2				SS
Michigan	3			1		LS
Minnesota	2					LS, P
Missouri	5					PS, LS, P
Mississippi		1				P
North Carolina				2		AS, SS
North Dakota	1					P
New York	2					LS
Ohio	1					LS
Oregon		1				WS
Pennsylvania		4				AS
South Dakota	1	2				PS, P
Saskatchewan	1					LS
Texas	5					P
Washington		1				WS
Wisconsin	8	1				LS
West Virginia	1					P
Totals	39	18	4	9	1	

Table 2. Number of National Sturgeon and Paddlefish Surveys returned from each state apportioned by type of agency. The species reported from each state are coded as: AS=Atlantic sturgeon, GS=Gulf sturgeon, GnS=Green sturgeon, LS=Lake sturgeon, SS=Shortnose sturgeon, ShS=Shovelnose sturgeon, PS=Pallid sturgeon, WS=White sturgeon, and P=Paddlefish.

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Results

The national survey of federal and state agencies, private growers, and universities was conducted from June 1995 to March 1996 with 71 completed surveys returned. All United States Acipenseriformes species were represented in the completed surveys: 6 – Atlantic sturgeon (*Acipenser oxyrhynchus*), 3 – Gulf sturgeon (*Acipenser oxyrhynchus*), 1– Green sturgeon (*Acipenser medirostris*), 1 – Shovelnose sturgeon (*Scaphirhynchus platorynchus*), 19– Lake sturgeon *(Acipenser fulvescens)*, 25 – paddlefish (*Polyodon spathula*), 4– Pallid sturgeon (*Scaphirhynchus albus*), 4 – Shortnose sturgeon (*Acipenser brevirostrum*), and 8 – White sturgeon (*Acipenser tansmontanus*). Surveys were completed by federal and state agencies, private growers, and universities/colleges from 26 states and 3 Canadian provinces (Table 2). Most surveys received were incomplete due to non-availability of information for some questions. Of 71 surveys returned, the information covered only 32 broodstock (45%), 38 hatchery reports (54%), and 35 field reports (49%) (Table 3).

Species	Number of surveys	Surveys sections Broodstock	Hatchery	Field
Atlantic sturgeon	6	5	1	2
Green sturgeon	1	1		
Gulf sturgeon	3	2	2	2
Lake sturgeon	19	9	11	10
Paddlefish	25	7	16	12
Pallid sturgeon	4	1	2	3
Shortnose sturgeon	4	3		2
Shovelnose sturgeon	1			1
White sturgeon	8	4	6	3
Totals	71	32	38	35

Table 3. Number of National Sturgeon and Paddlefish Surveys returned by species and types of information provided.

Broodstock information was reported on all 9 target species, identifying broodstock location, spawning period, and average age at maturity (Table 4). Surveys provided information on pairing criteria at spawning (40%), diet information (34%), hormones used to induce spawning (46%), egg de-adhesion methods (56%), incubation methods (50%), and tagging systems used to identify spawners (63%). Thirty-four percent of the broodstock had been genetically characterized by allozyme or DNA analysis.

Hatchery performance was reported in 38 surveys. Requested water quality information to characterize the production environment was provided for temperature, pH, and dissolved oxygen in 47% of reports while gas saturation, salinity, and alkalinity was provided in only 21% of reports. Most respondents reported cultural performance (84%) and production feed type (79%) Rearing unit types reported were raceways 14 (36%), tanks 13 (34%), ponds 9 (24%), vats 1 (3%), and cages 1 (3%). The average rating of species for six stressors using a 5 point scale (5 being most tolerant) was white sturgeon 3.8, Atlantic sturgeon 3.4, lake sturgeon 3.4, Gulf sturgeon 2.6, paddlefish 2.6, and pallid sturgeon 2.4. Special handling and transportation consideration were identified for 19 of 38 (50%) with paddlefish broodstocks most susceptible to handling stress. Disease considerations were identified in 18 of 38 reports: bacterial infections 17, parasites 9, fungus 3, and viruses 3.

Field performance information was reported on 34 surveys. Broodstocks were commonly reported to be best suited for wild stock enhancement in riverine environments. Pallid sturgeon were reported to be poorly adapted for lake or reservoir fisheries. A special habitat preference was listed on 56% of the questionnaires. Additional comments on performance and behavioral characteristics were provided on 23 (68%) reports.

Species/Stock	State/Province	Broodstock Location	Spawn Season Start	End	Age of Maturity Male	Female
Atlantic sturgeon						
Cape Fear River	NC	Cape Fear River				
Delaware River	PA	NE Fishery Center				
Hudson Estuary	PA	NE Fishery Center	May	August	14	15
Hudson River (Domestic)	PA	NE Fishery Center				
New Jersey Coastal	PA	NE Fishery Center				
Green sturgeon						
Klamath River	CA	Klamath River				
Gulf sturgeon						
Swannee River	FL	Swannee River	February	June	7	8
Swannee River	FL	Welaka NFH				
Lake sturgeon						
Black Lake	MI	Black Lake	May	June		
Flambeau River	WI	Flambeau River	May	May		
De Praires River	NY	Moon Lake				
Little Fork	MN	Rainy River	May	June	16	25
Menominee River	WI	White Rapids Stretch	April	May	25	15
Saskatchewan River (Wild)	SK	Saskatchewan River	May	June		
Sturgeon River	MI	Sturgeon River				
Wolf River	WI	St. Louis Bay				
Yellow Lake	WI	Yellow Lake				
Pallid sturgeon						
Missouri River	SD	Missouri River	May	June	8	10
Shortnose sturgeon						
Cape Fear River	NC	Cape Fear River				
Connecticut River	MA	Connecticut River	April	May	5	8
Merrimack River	MA	Merrimack River				
White sturgeon						
Kootenai River (Wild)	ID	Kootenai River	May	June	15	24
Lower Fraser River	BC	Malaspina University	April	July	15	17
UC Davis	CA	UC Davis	February	June	4	8
Paddlefish						
Alabama River	GA	Owen & Williams Fish Farm				
Cumberland/Ohio River	KY	Ohio River	April	May		
Gavins Point - BP	MO	Blind Pony Hatchery	March	May		
Gavins Point - SP	MN	St. Paul Area				
Mermentau	LA	Mermentau River	February	March	5	10
Missouri River (Wild)	SD	Missouri River	May	June	8	11
Table Rock Reservoir	MO	Table Rock Reservoir	Mar	Apr	6	10

Table 4. Spawning and ages of maturity of broodstocks of eight Acipenseriformes species reported in 1995 National Sturgeon and Paddlefish Survey.

Management applications

Managers today are asked to manage a diversity of fisheries (natural fisheries, restoration fisheries, and enhancement fisheries) in river, lake, and reservoir situations. The past practice of planting fish

from a single broodstock source into all waters has proven to be inefficient and can be destructive in fisheries with naturally reproducing broodstocks (Krueger et al., 1981; Kapuscinski and Jacobson, 1987). Hybridization of natural broodstocks with fish planted from other sources can result in genetic introgression and lead to possible population decline and ultimate loss of the adapted broodstock. Development of individual broodstock characteristics information, made freely available, allows managers to identify the broodstocks best adapted to particular fisheries and management situations. Managers can use information on fish performance, health status, genetic history, and availability to choose the specific broodstock to plant in each fishery. Commercial growers can choose broodstocks with characteristics that more closely match markets for their products, whether the fish are destined for stocking programs or food consumption. General availability of standardized broodstock information to fisheries managers and to the aquaculture industry will greatly increase the managers ability to match broodstock performance characteristics with the fishery.

The NFBR-SP is an initial attempt to develop comparable information on potential sturgeon and paddlefish broodstock sources in the United States. Standardized information will assist managers to identify broodstocks with genetic background, performance characteristics, and habitat preferences that best match the habitat where fish are to be stocked in management programs (supplementation or restoration) or marketed for consumption. Currently, the database is limited by incomplete information and non-reporting of some broodstocks, but will increase in value as the data set is completed and expanded.

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MOVEMENT AND HABITAT USE OF PADDLEFISH

IN THE UPPER MISSISSIPPI RIVER AND SELECTED TRIBUTARIES

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Dredging, flow modification, and the construction of dams and reservoirs on large river systems such as the Mississippi River have altered traditional paddlefish *Polyodon spathula* habitat and may impede paddlefish movements. Consequently, paddlefish have been extirpated from four states and Canada; furthermore, 9 of 22 states within the species range have reported significant declines in paddlefish populations. Paddlefish are listed as endangered or threatened in six states and are listed as a species of special concern by the U.S. Fish and Wildlife Service. Restoration or protection of depleted paddlefish populations is a goal of several state and federal natural resource agencies in the upper Mississippi River basin in the United States. Knowledge of paddlefish movements and habitat use is important for determining the effects of dams on paddlefish and for defining the spatial scale necessary to effectively manage depleted paddlefish stocks.

From November 1994 until March 1996, we evaluated movement and habitat use of paddlefish in the upper Mississippi River and two tributary rivers. In fall 1994 and spring 1995, paddlefish were captured in Pool 8 on the main stem of the upper Mississippi River; in the Wisconsin River downstream of a hydroelectric dam; and in the Chippewa River. We implanted 75 or 150 gram radio transmitters broadcasting at 49 MHz into the peritoneal cavities of these fish. Radio-marked paddlefish were located two to four times monthly from the time of receiving an implant until March 1996.

For most of the year, radio-tagged paddlefish stayed within a 1 to 4 km reach of the river that included the location at which they were captured. Habitats used by fish within these reaches was characterized by deep water and low current velocity as compared to other areas within these reaches. In spring 1995, about half (25 of 56) of the radio-tagged paddlefish migrated from their tagging locations, presumably for spawning. At each study site, this migration occurred when water temperature was near 10° C and discharge was high. Paddlefish in the Chippewa and Wisconsin Rivers migrated downstream from 20 to 90 km. Six of the 12 radio-tagged paddlefish in the Chippewa River migrated to Pool 4 of the main stem of the upper Mississippi River. Eleven of 24 paddlefish tagged in the

Wisconsin River migrated downstream within that river but did not enter into the Mississippi River. Paddlefish in Pool 8 of the Mississippi River remained in the upper portion of that Pool throughout the spring. Only 3 of 20 paddlefish tagged in Pool 8 made significant movements during spring; these 3 paddlefish moved upstream about 5 km to a navigation dam. Paddlefish that migrated during spring did not pass through any dams. At each study area, most paddlefish that migrated during spring returned to the river reach near their capture location by early summer.

In early fall 1995, 7 paddlefish moved long distances (90 to 250 km) from their capture site. This movement occurred when discharge in the main stem of the upper Mississippi River and its tributaries was high due to a period of high precipitation. Three paddlefish from the Chippewa River moved downstream about 90 km into the Mississippi River; one fish continued downstream, moving through a navigation dam into Pool 5 of the Mississippi River. All three of the paddlefish that moved from the Chippewa River returned to that river in late fall. One paddlefish radio-tagged in the Wisconsin River migrated downstream about 100 km into Pool 10 of the Mississippi River in early fall. As well, 3 paddlefish radio-tagged in Pool 8 of the Mississippi River moved downstream about 150 km to Pool 10; these fish moved through 2 navigation dams. One of these fish then moved up the Wisconsin River about 100 km.

In summary, paddlefish radio-tagged in the upper Mississippi River and its major tributaries spent most of the year in a 1 to 4 km reach of river which included the site at which they were radio-tagged. The habitat at fish locations within those 1 to 4 km reaches was characterized by deep water and low current velocity as compared to other habitat within those reaches. Paddlefish from all three radio-tagging sites made long (20 to 250 km) spring and fall migrations. These migrations occurred during periods of high discharge. Most of the paddlefish that participated in the spring and fall migrations returned to the reach of the river from which they were captured within a few weeks. During migrations, navigation dams on the upper Mississippi River may have impeded upstream movement of paddlefish more so than downstream movement. In our study 5 paddlefish moved downstream through navigation dams, whereas only 1 paddlefish moved upstream through a navigation dam. Our study suggests that large spatial scales are appropriate for managing depleted paddlefish stocks in the upper Mississippi River.

HABITAT SUITABILITY INDEX FOR LAKE STURGEON, *Acipenser fulvescens*

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Introduction

Since 1993, Ontario Hydro, Toronto, Ontario, Canada, has undertaken a study to develop a Habitat Suitability Index (HSI) model for lake sturgeon, in order to assist in efforts to protect and conserve this unique, long-lived species. In particular, this model was specifically designed to assist Ontario Hydro in managing lake sturgeon populations, especially on rivers with existing hydroelectric dams with their related operations, and during any redevelopment of existing stations and new developments.

In Ontario, lake sturgeon are largely confined to freshwater riverine and lacustrine habitats, although a few populations are found in the brackish estuarine waters of Hudson and James Bay. This fish species in considered to be common in fish communities in the northern areas of the province and in the Lake St. Clair area of southwestern Ontario. In fish communities elsewhere in the province, lake sturgeon are regarded as rare.

As the model has been constructed from the author's experience and data on lake sturgeon, from the existing literature, as well as from personal communication from many North American researchers, this model is expected to be applicable to large, slow flowing rivers within the native range of the species.

The structure of the lake sturgeon model is based upon measurable habitat variables. These habitat variables were considered to be important in limiting the distribution, abundance and/or survival of this species within its native range. Knowledge of critical habitat requirements is limited for the early life stages, including juveniles, and may require further model refinement as data becomes available.

HSI Model

Two model components were used to represent two life requisites of the lake sturgeon. They are: food (summer foraging habitat); and, reproduction (spawning and incubation habitat). Variables were selected for each of these components to represent direct or indirect measures to evaluate the characteristics of a site in meeting lake sturgeon habitat requirements for summer foraging and spawning.

Four habitat variables were selected for foraging, these being: foraging substrate type for adults (V_1); foraging substrate type for juveniles (V_2); water depth (V_3); and water velocity (V_4). Likewise, four variables were chosen as indicators of habitat suitability for spawning and incubation, these being: water temperature (V_5); water velocity (V_6); substrate type (V_7); and, water depth (V_8). The structure of the HSI can be described as follows:

HABITAT VARIABLES   **LIFE REQUISITE**

Substrate Type - Adult (V_1)

Substrate Type - Juvenile (V_2) ➤ FOOD

Depth - Juvenile (V_3)
Velocity - Juvenile (V_4)

OVERALL HSI

Temperature (V_5)

Velocity (V_6) ➤ REPRODUCTION

Substrate Type (V_7)

Depth (V_8)
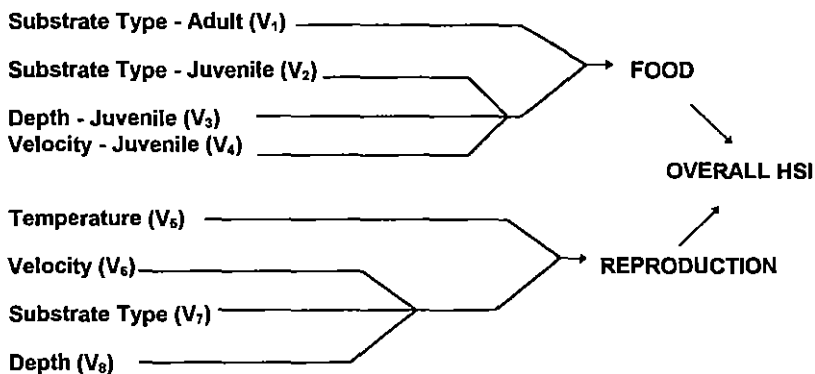
Suitability Index (SI) graphs for the eight model variables were constructed by converting all habitat information collected into habitat suitability graphs ranging from 0 (ie, unsuitable habitat) to 1 (ie, optimum habitat). The rationale and assumptions used in the development of the HSI model for lake sturgeon have also been addressed.

Ontario Hydro is presently undertaking field research to validate the precision of this model, as well as collecting new information to strengthen the model's predictions.

The model will be made available, on a laptop computer, for use by all in attendance at the Symposium. Real or simulated habitat variables can be employed.

Genetics

POPULATION GENETICS OF ATLANTIC STURGEON *ACIPENSER OXYRINCHUS*

BASED ON ANALYSIS OF MITOCHONDRIAL DNA

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Introduction
In what may be the largest genetic study of any acipenseriform, we surveyed mitochondrial DNA (mtDNA) variation in more than 600 specimens of *Acipenser oxyrinchus* (Atlantic sturgeon). Problems investigated included the relationships between the two forms that occur on the Atlantic and Gulf of Mexico coasts and also between *A. oxyrinchus* and *A. sturio* (European sea sturgeon), the stock structure of populations along the Atlantic and Gulf of Mexico coasts, the stock composition of the New York Bight commercial fishery, and gene flow among Gulf of Mexico stocks.

Taxonomic Investigations
In the U.S., the taxonomic status of threatened or endangered populations of vertebrates is important in determining the degree of protection they may receive under the Endangered Species Act (O'Brien & Mayr 1991). For *Acipenser oxyrinchus*, the Atlantic (*A. o. oxyrinchus*) and Gulf forms (*A. o. desotoi*) were designated subspecies based on differentiation of morphological features, although only two Gulf specimens were examined (Vladykov 1955). Wooley (1985) reanalyzed these morphological features of a larger sample of Gulf sturgeon and concluded that only one, relative spleen length, was diagnostic. However, values for relative spleen length of the two forms were not discrete and it may be that differences in these values are ecophenotypic in nature. We used direct sequencing of a hypervariable area (203 base pairs) of the control region of mtDNA to quantify the extent of genetic differentiation between the two putative subspecies (Ong et al. 1996). Representatives of each subspecies from populations across their distributions were surveyed and three fixed differences were found among 15 polymorphic sites. An additional two nucleotides were nearly fixed. Although polymorphisms also were detected within populations across the distribution of each subspecies, no fixed differences among populations were found. Ong et al. (1996) concluded that these data strongly supported the designation of subspecies of *A. oxyrinchus*.

The relationship between *A. oxyrinchus* and *A. sturio* has long been debated. These fish are found on opposite sides of the North Atlantic and are phenotypically very similar. *Acipenser oxyrinchus* occurs widely along the Atlantic coast of North America. *Acipenser sturio*, which

once had a similarly broad range in Europe and Asia, now occurs only in very low abundances in both the Gironde River, France, and the Black Sea (Rochard et al. 1990). Some workers have considered the Atlantic sturgeon to be synonomous with or a subspecies of *A. sturio* (Scott & Scott 1988; Birstein 1993), but Vladykov and Greeley (1963) and Magnin (1964) recommended they be given separate specific status, pending additional research. We obtained a tissue sample from one *A. sturio* specimen captured during 1994 in the Gironde River. We then compared a portion (203 bp) of the control region of mtDNA between *A. sturio* and *A. oxyrinchus*.

We found a minimum of 31, and a maximum of 33 nucleotide substitutions between the individual of *A. sturio* and more than 70 individuals of both subspecies of *A. oxyrinchus*. Three sites also exhibited nucleotide additions or deletions. In comparison, the number of nucleotide substitutions between any pair of specimens of the two subspecies of *A. oxyrinchus* ranged between five and eight, with no additions or deletions. Excluding additions or deletions, nucleotide divergence between *A. oxyrinchus* and *A. sturio* was approximately 15%, much higher than the maximum of 3.5% between the two subspecies of *A. oxyrinchus* (Ong et al. 1996). There are no unambiguous criteria for the interpretation of molecular data in determining taxonomic status (e.g., O'Brien & Mayr 1991, Wayne 1992). However, we believe that the level of differentiation observed argues strongly for full species status of each of the western and eastern Atlantic sea sturgeons.

Stock Structure Along the Gulf of Mexico
Populations of *A. oxyrinchus* occur in drainages of the Gulf of Mexico and along the Atlantic coast of North America. *Acipenser oxyrinchus desotoi* are considered threatened by U.S. Fish and Wildlife Service. Historically, *A. o. desotoi* were found in major river systems extending from central Florida to the Mississippi River; many of these drainages still host depleted populations (USFWS & GSMFC 1995). Efforts are being considered to restore depressed populations through hatchery supplementation. However, knowledge of the stock composition of *A. o. desotoi* was federally mandated prior to the initiation of restorative efforts, so that native gene pools are preserved. The Suwannee River population has been extensively studied over the past decade and probably contains the largest population of sturgeon along the Gulf of Mexico. Miracle & Campton (1995) examined the extent of genetic variation in sturgeon from the Suwannee River to determine if its population constituted a single homogeneous unit. Sequence analysis of 268 base pairs of a highly variable area of the mtDNA control region did not reveal significant genetic heterogeneity among sturgeon from different sampling locations.

We used restriction fragment length polymorphism (RFLP) and sequencing analyses of mtDNA to assess the stock structuring of *A. o. desotoi* populations among eight drainages extending from the Mississippi River to the Suwannee River (Stabile et al. 1996). RFLP analysis using four diagnostic restriction enzymes yielded eight composite haplotypes; genotypic diversity indices (Nei & Tajima 1981) ranged between 0.173 for the Choctawhatchee River sample to 0.732 for the Yellow River collection. Significant differences ($P < 0.05$) in haplotype frequencies indicated substantial geographic structuring of sturgeon populations; results from the RFLP ($N = 164$) and sequence ($N = 141$) analyses were largely congruent. Five regional or river-specific stocks were identified (from west to east): (1) Lake Ponchartrain and Pearl River, (2) Pascagoula River, (3) Escambia and Yellow rivers, (4) Choctawhatchee River, and (5) Apalachicola, Ochlockonee, and Suwannee rivers. These results suggest strong reproductive isolation of *A. o. desotoi* stocks on at least a regional basis, and point to the inadvisibility of mixing of hatchery-reared progeny of broodstock from different Gulf rivers.

Stock Structure Along the Atlantic Coast

We used RFLP analysis of mtDNA with five diagnostic restriction enzymes to characterize the stock structure of populations of *A. o. oxyrinchus* along the Atlantic coast, including the St. Lawrence River, Quebec; St. John River, New Brunswick; Hudson River, New York; Edisto River, South Carolina; and four rivers in Georgia; the Altamaha, Ogeechee, Savannah, and Satilla (Waldman et al. 1996 a,b). Chi-square analysis showed the eight populations could be grouped as three highly differentiated (P < 0.0001) stocks: (1) Canadian (St. Lawrence and St. John rivers); (2) Hudson River; and (3) southeastern (Edisto, Savannah, Ogeechee, Altamaha, and Satilla). Composite haplotypes showed a clear cline in genotypic diversity indices (Nei & Tajima 1981) among populations that ranged from complete monomorphism (0.0) of the two Canadian populations to considerable polymorphism among southeastern populations (e.g., Edisto River: 0.646; Ogeechee River: 0.750). A latitudinal cline in genotypic diversity along the Atlantic coast is consistent with founder effects among northern populations that recolonized glaciated drainages from more genotypically diverse populations in southern, nonglaciated regions.

Mixed-Stock Analysis of the New York Bight Fishery

For some wide ranging species, fisheries have developed distant from spawning and nursery areas, and these fisheries may harvest individuals from more than one stock. For management purposes, it is important to quantitatively estimate the relative contributions of individual stocks to mixed fisheries to allow managers to protect threatened stocks at sites distant from their natal rivers. Successful application of genetic approaches to mixed-stock analysis is dependent on the existence of significant differentiation of genetic characters among spawning stocks which contribute to the mixed fishery (Utter & Ryman 1993, Xu et al. 1994). To conduct mixed stock analysis, frequencies of genotypes must be characterized in reference spawning stocks and in the mixed fishery.

A targeted coastal fishery for *A. o. oxyrinchus* has developed in recent years along the mid-Atlantic coast of New Jersey and New York (New York Bight). Waldman et al. (1996a) performed RFLP analysis of specimens of *A. o. oxyrinchus* from eight populations from Canada to Georgia and concluded based on haplotype frequency differences that three statistically discrete (P < 0.0001) stocks exist: (1) Canadian, (2) Hudson River, and (3) southeastern. Haplotypic frequency data of these stocks was then used in a mixture model (constrained least squares; Xu et al. 1994) to estimate the relative contributions of each of these stocks to a sample of Atlantic sturgeon (N = 112) from the fishery in the New York Bight off New Jersey. This analysis showed a 97% to 99% contribution from the Hudson River stock, with the remainder from the southeastern stock. The overwhelming contribution of the Hudson River stock was attributed both to (1) a hypothesized tendency for marine migrating Atlantic sturgeon to remain within the geographic provinces of their natal rivers (the Hudson River is within the Virginian province), and (2) to the absence of other robust Atlantic sturgeon populations within the Virginian province.

Gene Flow and Homing Fidelity

Most populations of sturgeons are anadromous or potamadromous and thus, migrate from marine or lake waters to rivers to spawn. However, almost nothing is known of the degree of homing fidelity shown by acipenseriforms. Although homing fidelity of fishes may be studied directly by means of mark-recapture (e.g., Melvin et al. 1986), the relative scarcity and high value of sturgeons precludes such an approach. An alternative is to assess homing fidelity indirectly through genetic analysis (Tallman & Healey 1994).

Homing fidelity of sturgeons through genetic analyses would best be assessed among populations in rivers that drain to a common water body and that historically have a stable geographic history to avoid confoundment by founder effects as a consequence of recolonizations. Stabile et al. (1996) used both RFLP and sequencing analysis of mtDNA to estimate gene flow among five stocks of *A. o. desotoi* that occur in eight drainages that feed the Gulf of Mexico between Mississipi and Florida. The five stocks were defined based on χ^2 analyses ($\underline{P}$ < 0.05) of haplotype frequencies; some stocks were equivalent to single populations, whereas others were regional stocks made up of two or more populations. Pairwise gene flow estimates ($\underline{N}_m$) between stocks were derived from $\underline{F}_{st}$ values (Wright 1943) obtained via AMOVA analysis (Excoffier et al. 1992).

Pairwise estimates of gene flow (Table 1) among the Gulf stocks based on sequencing analysis ranged from 0.15 between the western (Lake Ponchartrain and Pearl River) stock and the Escambia River-Yellow River stock, to 1.2 between the Escambia River-Yellow River stock and the eastern stock (Apalachicola, Ochlockonee, and Suwannee rivers). Gene flow estimates derived from RFLP analysis were even lower on average, and ranged from 0.09 between the western and Choctawhatchee River stocks to 0.66 between the western and Escambia River-Yellow River stock.

These gene flow values are very low in comparison with estimates for other anadromous fishes. Estimated annual straying rates among populations of Pacific salmons have ranged between about 1% and 27% (reviewed in Adkinson 1996). Laughlin & Turner (1996) used two statistical methods to estimate N_m of *Morone saxatilis* (striped bass) among three Virginia tributaries of Chesapeake Bay; the private allele approach of Barton & Slatkin (1986) yielded an estimate of N_m = 14.2, whereas the F_{st} approach yielded an estimate of N_m = 2.7. In a mark-recapture study, Melvin et al. (1986) estimated an annual straying rate of 3% among Canadian populations of *Alosa sapidissima* (American shad).

Moreover, the low gene flow estimates for *A. o. desotoi* were obtained across populations that occur in eight rivers, the mouths of which are arrayed across little more than 500 km of coastline. Sturgeon from these rivers have the opportunity to mix in the Gulf of Mexico during winter. These mtDNA data show that despite the geographic proximity of these rivers, stocks of *A. o. oxyrinchus* generally exchange less than one female per generation, a level sufficient to permit genetic differentiation at the stock level (Adkinson 1996). Gene flow estimates also were generally higher among proximal stocks, suggesting that what straying occurs does so in 'stepping stone' fashion (Kimura & Weiss 1964) in which migrants among semi-isolated populations are exchanged chiefly with neighboring populations. If this is true for *A. o. desotoi*, then such spatially restricted straying should have contributed to the geographic structuring observed among these populations (Adkinson 1996). Stabile et al. (submitted) hypothesized that the homing imperative of *A. o. desotoi* for spawning purposes is strong, but that it may be reinforced by metabolic constraints. *Acipenser oxyrinchus desotoi* return to rivers from the Gulf of Mexico to summer near cold water springs; tagging has shown that individuals are recaptured at the same cool water refuges in which they were first tagged (Clugston et al. 1995). Low gene flow estimates indicate that natural recolonization of populations of *A. oxyrinchus* would proceed slowly.

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INHERITANCE OF MICROSATELLITE LOCI IN LAKE STURGEON

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Introduction

Lake sturgeon (*Acipenser fulvescens*) were at one time abundant throughout the Great Lakes drainage but overharvesting, pollution, and the damming of rivers have pushed the sturgeon's abundance to low levels. For example, in Lake Erie, the initial commercial harvest of sturgeon was 2,250,000 kg in 1860 but by 1895 this level had dropped by 80% (Scott and Crossman 1973). Populations that still remain today generally exist at low levels and exploitation is restricted to a few populations in Wisconsin and Canada.

The genetics of sturgeon are poorly understood for several reasons. The first is that the life history of sturgeon make genetic investigations difficult. Sexual maturation in sturgeon requires 10-30 years and then spawning is intermittent (1-6 years). Another problem is the difficulty in capturing an organism that lives on the bottom of large lakes and rivers. Current techniques to study fish genetics generally rely on allozymes which require the sacrifice of the fish in order to sample their internal tissues. Thus, allozymes require the mortality of individuals from already threatened populations. Genetic studies are further complicated by their octoploid nature (Blacklidge and Bidwell 1993).

Development of microsatellites as genetic markers for lake sturgeon would facilitate genetic studies. Microsatellites can be used as a genetic marker that requires non-lethal tissue sampling. Microsatellites often provide greater discriminatory power than allozymes, especially if there are low levels of gene flow. These markers have already been shown to be useful in genetic studies of brown trout (Estoup et al. 1993). If the markers are shown to work for lake sturgeon, they will provide a useful tool for examining the genetics of lake sturgeon at a population level. Population genetics data are particularly needed for the development and implementation of species restoration projects.

A restoration effort involving lake sturgeon is being undertaken by the Menominee Indian Tribe of Wisconsin. Lake sturgeon were historically used by the Menominee people and are important culturally and spiritually to the Menominee (Beck 1993). The fish are blocked by dams from returning to traditional spawning sites, particularily Keshena Falls on the Wolf River, and so the tribe currently has no access to lake sturgeon. As a result, a multi-agency Menominee Reservation Sturgeon Management Planning Committee was formed and includes members from the Menominee Tribe, the Wisconsin Department of Natural Resources, the Bureau of Indian Affairs, and the U.S. Fish and Wildlife Service. The committee adopted the management goal of establishing a self-sustaining population of lake sturgeon within waters of the reservation. In 1994, the Planning Committee drafted the "Menominee Reservation Lake Sturgeon Management Plan" and initiated efforts to re-establish lake sturgeon. The plan identifies genetics as being important to the success and health of establishing a population. This research should provide a tool to address this issues.

In a previous study, a genomic library of lake sturgeon DNA was screened to identify tri- and tetrameric repeat motif microsatellite loci. A screen of 60,000 clones identified twelve primer pairs that amplified microsatellite loci that produced products in most North American sturgeon species (May et al., in review). This paper describes the segregation of these loci in single pair matings (families) of lake sturgeon to describe their inheritance applicability for population studies.

Methods

Sample collection: Fish from the Menominee River in Wisconsin were collected below the White Rapids dam using a Wisconsin Department of Natural Resources (WIDNR) electroshocking boat . A portion of fin was sampled from each fish. Half of the fin clip was stored in 100% alchohol and the other half placed in lysis buffer (150 mM EDTA, 50 mM Tris ph 8.0, 2% n-lauroylsarcosine) for later extratction of DNA. Adults in spawning condition when captured were held in 1.8 meter diameter tanks to let them spawn on their own. This technique was used successfully in spring 1994 (Thuemler, personal communication). Tanks were examined every day to determine whether the fish were ready to spawn. Milt was collected from three males using a syringe and stored on ice. Eggs were then stripped from a single female into separate containers to create three separate families when fertilized with milt from the different males. The fertilized eggs were held in the Wild Rose, Wisconsin hatchery for several days before being transported to the hatchery at Cornell University. All fry from the Menominee River families (N=14,20,30) were sampled at hatch because the hatch was poor due to a fungus problem. Similarily, seven families were made from two females and four males captured in the St. Lawrence river at Montreal, Quebec. One hundred fry from each St. Lawrence River family were sampled with 50 being frozen and stored in an ultra-cold freezer and 50 stored in lysis buffer.

DNA extraction: DNA extraction was done using a CTAB extraction described by Grewe et al. (1993), with the following modifications: 30 ug

of proteinase K was used in each sample and the samples were incubated overnight at 55° C

PCR conditions: The 50 ul reaction contained 1ul of genomic DNA (20 ng), 5 ul 10X PCR buffer (Gibco BRL), 1.5-2.5 ul 50mM $MgCl_2$, 1 ul each of 20uM forward and reverse primer, 2.0-3.5 (ul 2.5mM dNTPs), 0.2 ul (5 U/ul) Gibco*Taq* polymerase, and 35.8-38.3 ul H_2O. PCR was performed in a Perkins-Elmer DNA Thermocycler. Thirty-five cycles were run as a 3 step function. The first step was a denaturing step at 94° C for 1 minute. The second step was an annealing step at 57° C for 30 seconds. The final step was an extension step at 72° C for 30 seconds. The initial denaturing step was run for 3 minutes and the final extension step was run for 5 minutes.

Gel conditions: Samples were run on a 4% Metaphor^R gel at 460V with the 0.5 TBE gel buffer run through a Neslab RTE 100 cooling unit to keep the temperature at 20° C. In a pre-run step, the gel was run for 5 minutes at 460V in $12\text{-}13^{\circ}$ C buffer. The voltage was then turned off until the buffer was brought up 20° C and then restored and the gel run for 1-1.5 hours (run time was determined by the clone size based on 15 minutes for every 25 base pairs). Gels were stained with ethidium bromide and examined under UV light. A polaroid picture was taken and used for scoring individuals.

Results and Discussion

Polymorphisms were found in five of the twelve loci examined. The loci that were polymorphic are LS-19, 34, 39, 54, and 68. LS-19, 34, and 54 exhibit two alleles, LS-39 three alleles, and LS-68 four alleles. The three monomorphic loci include LS-58, 62and 73. Loci LS-22, 23, 57, and 69 either did not amplify well or did not resolve well enough to be used further. Table 1 outlines some of the specifications for the loci used.

Table 1. Locus designation with size, repeat motif and current status.

Locus	Clone Size	Repeat Motif	Status
LS-19	133	(TTG)9	Polymorphic
LS-22	218	(AAAT)6 (AAG)30	Not resolved
LS-23	162	(GTTT)8	Not resolved
LS-34	137	(GTT)10	Polymorphic
LS-39	129	(GTT)10	Polymorphic
LS-54	177	(GATA)6 (GACA)7	Polymorphic
LS-57	206	(GAA)29	Not resolved
LS-58	198	(GATA)20	Monomorphic
LS-62	85	(GATA)7	Monomorphic
LS-68	120	(GATA)13	Polymorphic
LS-69	206	(TATC)13	Not resolved
LS-73	214	(ACTC)12	Monomorphic

Polymorphic loci are currently being examined to determine the mode of inheritance. Preliminary results suggest the polymorphic loci exhibit tetrasomic inheritance. Evidence suggesting tetrasomic inheritance includes gene dosage in loci LS-19,34,39 and 54; see Figs. 1 and 2 and note assymetric phenotypes in LS-19 and LS-54 and three alleles present in progeny of a LS-68 cross from parents that exhibit three alleles (Fig. 3).
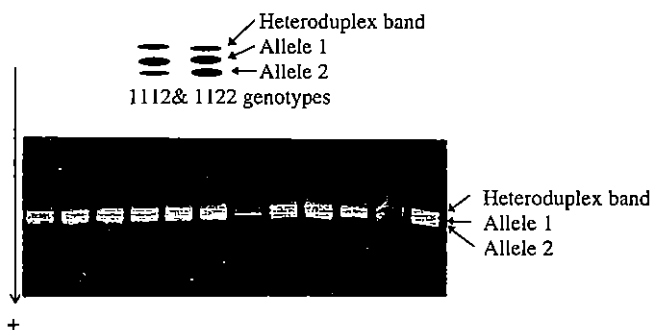
Figure 1. LS-19 cross of a 1222 male X 1111 female with progeny showing genotypes 1112 (lanes 1-7 and 10) and the 1122 (lanes 8, 9 ,11 and 12). All individuals exhibit a heteroduplex band as the upper band. The homoduplex bands are under the heteroduplex band with the upper band being designated the 1 allele.
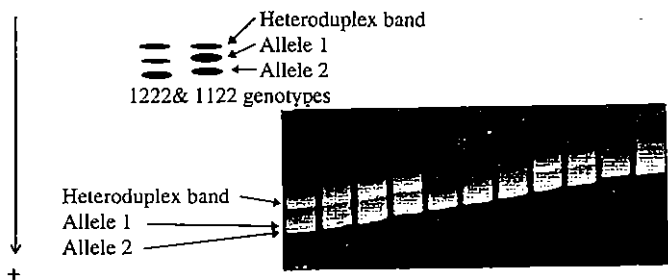
Figure 2. LS 54 cross of a 1122 male X 1122 female with progeny expressing genotypes of 2222(lanes 5, 6, and 7), 1122(lanes 1, 2, 4, and 8), 1222(lanes 3 and 9), and 1111(lane 10). Lane 11 is the LS-54 clone.
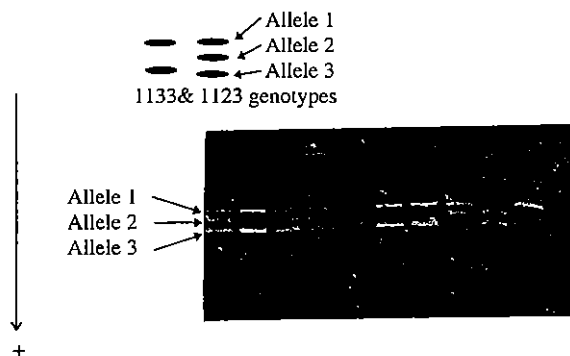
Figure 3. LS 68 cross of a 1111 male X 2233 female with individual progeny exhibiting all three alleles. Lanes 1, 3, and 5, are individuals exhibiting all three alleles. Lanes 2, 6, and 7 are individuals exhibiting alleles 1 and 3 and lane 8 is an individual with alleles 1 and 2. Lane 9 is the female's genotype and lane 10 is the male's genotype. Lane 5 was unresolved for this individual.

Additional progeny are being run to determine if Mendelian ratios shown in these crosses are consistent with tetrasomic inheritance. These results will determine if these genetic markers can be used in determining the population structure of remaining wild sturgeon stocks and as a tool to monitor the effects of transplanting and stocking of hatchery reared fish in the wild.

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INDUCTION OF MEIOTIC GYNOGENESIS AND POLYPLOIDY IN

WHITE STURGEON (*ACIPENSER TRANSMONTANUS* RICHARDSON)

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Abstract

White sturgeon (*Acipenser transmontanus*) eggs fertilized with untreated or UV-irradiated sperm (180-270 s, 1200 $\mu W/cm^2$) were subjected to either an early heat shock (32-34°C, 12-15 minutes post fertilization, 1-10 min duration), or an early cold shock (3°C, 12-15 minutes post fertilization, 15-180 min duration) to produce polyploids and meiotic gynogens. Control hatch rates ranged from 72-84%, compared with <1-21% for the gynogens. Ploidy analysis was performed on 2401 individuals using a Coulter Counter to analyze the particle size distribution of erythrocyte nuclei. Ploidy was confirmed for a subsample of 162 of these fish using flow cytometry. Efficiency of second polar body retention for the different temperature shocks ranged from 1-100%. In addition to the expected diploid and triploid fish, ploidy analysis of erythrocytes also revealed the existence of three tetraploid, six predominantly-haploid mosaic, and one diploid-tetraploid mosaic fish. The parental origin of gynogenetic, predominantly-haploid mosaic, and tetraploid fish was examined using sire and dam-specific Random Amplified Polymorphic DNA (RAPD) markers.

Introduction

Artificial induction of gynogenesis and polyploidy in sturgeon species is of interest to both commercial aquaculturists and researchers investigating the developmental biology and genetics of chondrostean fish. The specific mechanisms of sex determination in chondrosteans are not known, and the effects of ploidy manipulation on sex segregation ratios in sturgeon have not been described. The potential production of all female populations would be of obvious interest to sturgeon caviar producers. The objective of this investigation was to produce gynogenetic, triploid and diploid control white sturgeon to allow for the eventual examination of sex differentiation and segregation ratios in these groups of fish. The earliest work on the use of artificial techniques for the production of meiotic gynogenetic sturgeon was reported by Romashov et al. (1963) but in this study none of the gynogenetic larvae survived beyond 192 days post-hatch. Preliminary experiments on the induction of gynogenesis in white sturgeon failed to produce any viable gynogens (Kowtal, 1987).

Material and Methods

Gamete collection

Experiments were conducted at the U.C. Davis Aquatic Center in the Spring of 1995, using as a broodstock the first hatchery generation of white sturgeon raised in captivity. Spawning induction and gamete collection was as described by Conte et al. (1988). Each of four females (experiments 1 through 4, respectively) were induced to spawn by injections of 40 µg/kg body weight of the gonadotrophin-releasing hormone analogue [D-Ala^6, Pro^9-NEt]-GnRH. Males were induced to spermiate by a single injection of 1.5 mg/kg common carp pituitary extracts.

Experimental Design

Eggs were subjected to an early temperature shock (32-34°C, 1-10 min duration; or 3°C, 15-180 min duration) after fertilization with either untreated or UV-irradiated sperm to produce polyploids and meiotic gynogens, respectively (see Table 2). Temperature shocks were applied at 15 min post-fertilization in experiments 1 and 4, and at 12 min post-fertilization in experiments 2 and 3. All experiments included a diploid control group, a haploid control group to determine the efficacy of UV inactivation of the sperm (untreated eggs and UV-irradiated sperm), a triploid control group to determine the efficacy of second polar body retention (temperature shocked eggs and untreated sperm), and a treatment designed to induce gynogenesis (temperature shocked eggs and UV-irradiated sperm).

Sperm Treatment

For UV-irradiation, 2 ml of sperm stored in an atmosphere of pure oxygen at 4°C was diluted with 18 ml of seminal fluid (supernatant from surplus semen centrifuged at 6000 rpm for 20 min) and put into a rectangular pyrex dish (17 x 12 cm) to a depth of approximately 1 mm. This dish was placed on a gently rotating platform (90 rpm) 26-28 cm below two 15 W UVC bulbs (NIS G15T8) which provided an incident light intensity of 1200 µW/cm^2 as measured by the UVC probe of a UVX radiometer. The duration of UV-irradiation was adjusted for each batch of sperm such that the motility of the activated sperm was reduced to approximately 50%. Sperm was treated with UV irradiation for 180 to 270 seconds. At the completion of irradiation, 380 ml of 16°C water from the incubation system was added to the irradiated sperm suspension, and this mixture was immediately added to ova. The addition of the sperm suspension to the ova was considered to be the time of fertilization. Procedures following the UV-irradiation of the sperm were performed in the absence of visible light to prevent the possible photoreactivation of the sperm's DNA. A portion of each batch of untreated sperm was used to fertilize ova in the control diploid and polyploid treatment groups. The fertilization technique for these groups was as described except that 2 ml of undiluted sperm was added to 398 ml of incubation water immediately prior to fertilization.

Ova Treatment

Ova in coelomic fluid were maintained in a 4 l glass beaker at 16°C. For each treatment group 10-25 ml of ova were poured into a 190 mm diameter crystallizing dish and 400 ml of the appropriate sperm suspension was immediately added and the mixture was slowly stirred for three minutes. At this time the eggs were rinsed with 800 ml of fresh water four times to ensure that no viable sperm entered the incubation system. The eggs became sticky upon activation and they were quickly dispersed with a plastic transfer pipette to form a single layer across the bottom of the crystallizing dish. All dishes were kept at 16°C until the temperature shock. Heat shock treatments involved pouring the 16°C water off the eggs adhered to the bottom of the bowl, and immediately immersing the bowl in an oxygenated water bath set at the appropriate temperature. At the termination of the temperature shock the bowl was immediately immersed in a 15 l round fiberglass tank located within a semi-recirculating hatching system (16°C ± 0.5°C). Eggs that received a cold temperature shock were treated as described, using prechilled oxygenated water and a 3°C incubator for the duration of the cold shock treatment.

Verification of Gynogenesis

Gynogenesis was verified by the analysis of sire and dam-specific random amplified polymorphic DNA (RAPD) markers generated by the polymerase chain reaction (PCR) (Van Eenennaam et al., submitted). DNA was obtained from a single barbel which was rinsed in 200 µl sterile H_2O, and placed in a 500 µl eppendorf tube with 200 µl of 5% chelex, and 20 µg Proteinase K. The tube was then vortexed, incubated at 55°C for 2-3 hours, heated to 95°C for 12 minutes, and spun at 14,000 rpm in a microcentrifuge for 10 minutes. One hundred microliters of the clear supernatant was then removed and diluted 1:50 in 0.1X TE prior to use in the RAPD PCR reaction.

Verification of Polyploidy

Blood was collected from the caudal vein when the experimental animals were approximately 4 months old. The ploidy of blood cells was determined by nuclear volume analysis using a Coulter Counter Model ZM and 256 Channelyzer. In a subset of 162 fish, ploidy was determined by both blood cell nuclear volume and DNA content measurements to verify the accuracy of the Coulter Counter ploidy determination for this species.

Results

A total of 2401 samples were analyzed with the Coulter Counter and of these 2400 gave a reading which allowed for an unambiguous ploidy determination (Table 1). There was no overlap between the median channel data values observed for haploid, diploid, triploid, and tetraploid blood cell nuclei. Flow cytometry DNA content analysis of a random subset of 162 blood samples agreed with the Coulter Counter ploidy determination. Mosaic individuals with both predominantly-haploid (less than 35% diploid) and diploid-tetraploid blood cells were included in this concordantly classified subset. The mean flow cytometer channel data values for haploid, diploid, triploid and tetraploid erythrocyte nuclei corresponded to estimated nuclear DNA contents of 5.5, 10.7, 15.6, and 20.2 pg, respectively.

Table 1. Mean channel data from Coulter Counter and flow cytometry analysis of erythrocyte nuclei derived from diploid, triploid, tetraploid and mosaic white sturgeon.

COULTER COUNTER	Haploid mosaic	Diploid	Triploid	Tetraploid	Diploid-Tetraploid mosaic
Average	20.50/73.8	73.14	130.72	182.67	87/185
S.D.	1.38	6.32	9.01	8.99	---
Number	6	1127	1263	3	1
%	.25	46.96	52.63	.13	.04
Minimum	18	54	103	170	---
Maximum	22	93	159	190	---
FLOW CYTOMETER					
Average	25.5/47.9	49.46	72.15	---	52.7/93.7
S.D.	---	2.70	4.18	---	---
Number	1	71	89	---	1
Minimum	---	44.0	64.0	---	---
Maximum	---	55.4	81.7	---	---

Table 2. Neurulation and normal hatching frequencies, and ploidy analysis at 4 months by experimental treatment group in white sturgeon *Acipenser transmontanus* Richardson. Modified from Van Eenennaam et al., submitted. Aquaculture (Table 1).

Expt. (No.)	Design	Egg Treatment	Egg (No.)	Neurulation (%)	Hatch (%)	Ploidy (%) 2N	Ploidy (%) 3N	Other (No.)
1	Diploid	Untreated	940	93	72	100		
	Haploid	Untreated	703	1	0			
	Triploid	34°C, 1 min	836	93	45	55	44	1 2N/4N
	"	34°C, 2 min	770	93	30		100	
	"	34°C, 5 min	845	77	9	20	80	
	Gynogen	34°C, 1 min	758	8	< 1	100		
	"	34°C, 2 min	792	6	3			
	"	34°C, 5 min	977	8	< 1			
2	Diploid	Untreated	1489	88	74	100		
	Haploid	Untreated	1138	< 1	0			
	Triploid	3°C, 60 min	2244	43	17	41	57	4 N/2N
	Gynogen	3°C, 60 min	6405	< 1	< 1	100		
3	Diploid	Untreated	968	89	84	100		
	Haploid	Untreated	2588	< 1	0			
	Triploid	32°C, 2 min	1162	89	63	10	90	
	"	32°C, 3.5 min	1127	93	79		100	
	"	32°C, 5 min	1095	92	69	3	97	
	"	3°C, 15 min	1146	90	82	98	1	1 4N
	"	3°C, 30 min	1103	87	70	64	35	2 4N
	"	3°C, 60 min	1108	76	52	10	90	
	"	3°C, 180 min	1063	22	4	50	42	1 N/2N
	Gynogen	32°C, 2 min	1410	2	< 1	100		
	"	32°C, 3.5 min	1475	2	2			
	"	32°C, 5 min	1178	< 1	< 1			
	"	3°C, 15-30min	2749	5	< 1			
	"	3°C, 60 min	1290	6	4	100		
	"	3°C, 180 min	1274	< 1	< 1			
4	Diploid	Untreated	1574	87	74	100		
	Haploid	Untreated	1840	19	< 1	100		
	Triploid	34°C, 3 min	2255	87	53	29	71	
	Gynogen	34°C, 3 min	10631	26	21	98		1 N/2N

Triploid juveniles were phenotypically identical to the diploid fish, and no difficulties were experienced in raising them. The juvenile weight at 4 months of age was 39.6 ± 19.6 g (mean ± s.d, n = 1948), and no significant difference was found between diploid, triploid, and gynogenetic fish (data not shown). Gynogenetic larvae displayed a behavioral peculiarity in that they appeared to swim randomly throughout the water column and at the water surface while the diploid and triploid control larvae exhibited the "normal" behavior of aggregating on the bottom of the tank. This aberrant behavior persisted in the gynogenetic juveniles throughout the first 40 days but was not as pronounced thereafter. At 40 days post-hatch average survival was 87%, 84%, and 62%, in the experiment 4 diploid, triploid and gynogen treatments, respectively. The 68 surviving gynogens from the first three experiments , and 40 randomly-selected gynogens from experiment 4 were screened for the presence of the appropriate RAPD sire-specific markers. There was no evidence of paternal inheritance in 105 of these fish (20/20, 14/15, 31/33 and 40/40 from experiments 1, 2, 3, and 4 respectively).

Discussion

Measuring erythrocyte nuclear volume with a Coulter Counter and channelyzer is an indirect method to assess ploidy. We verified the accuracy of Coulter Counter ploidy determinations for this species in a subsample that was analyzed by flow cytometry, a direct method of ploidy determination. The ploidy determination data of the Coulter Counter and flow cytometer were in complete agreement and overall we found the Coulter Counter and channelyzer to be an inexpensive, rapid and accurate technique for ploidy determination in sturgeon.

When similar temperature shocks were applied to different batches of eggs we observed different rates of second polar body retention. The optimal timing of the temperature shock appeared to depend on the batch-specific stage of egg development. The stage of egg development at induced spawning may differ between individual females. A batch-specific treatment response was also seen with the male gametes. Identical UV-irradiation levels resulted in batches of sperm with differing abilities to initiate egg activation. The overall low number of gynogens produced in experiments 1-3 suggests that the sperm were unable to effect egg activation following UV-irradiation. This hypothesis is supported by the low rates of neurulation in the haploid control groups, and the observation that very few of the eggs receiving UV-irradiated sperm underwent even the first cleavage indicating that fertilization did not take place. The UV-irradiated sperm in Experiment 4 were more effective at egg activation, as evidenced by the 19% and 26% neurulation rates in the haploid control and gynogen treatment groups, respectively. Over 2000 putative gynogen hatched normally in the experiment 4 gynogen treatment group, and we will continue to rear several hundred through sexual differentiation. We are also rearing the 65 confirmed gynogens from experiments 1-3. Although our experiments were not designed to determine the optimal parameters for the induction of meiotic gynogenesis and polyploidy in white sturgeon, their results suggest that the optimal conditions may be dependent upon the unique developmental stage of the specific batches of gametes involved in each cross.

In the course of this experiment most of the embryos in the haploid control treatment groups died at an early stage of development before neurulation, or they displayed features of haploid syndrome (abnormal body shape, open blastopore) and failed to hatch normally. The six mosaics with predominantly-haploid erythrocytes that were detected in our ploidy analysis seemed to be fully viable, normal-appearing fish. Their weight at four months of age ranged from 15.33-26.86 g which was within the range of weights observed for the diploid and triploid fish in their respective treatment groups. RAPD analysis revealed that four of these predominantly-haploid mosaics had no apparent paternal inheritance (no analysis was done on the other two fish). The presence of a secondary population of diploid erythrocytes, and possibly the polyploid nature of white sturgeon may offer some explanation as to how these mosaic fish were able to tolerate the normally lethal gene dosage insufficiencies of the haploid condition.

Conclusion

We described the production of viable polyploid and gynogenetic white sturgeon. Although the effectiveness of our treatments to induce gynogenesis and second polar body retention was variable, the high percentage of verified gynogenetic and polyploid sturgeon in some progeny groups suggests that there is potential to use these techniques for further research and development in the domestic breeding of chondrosteans for aquaculture. We plan to continue rearing the gynogens produced in this study until gonadal sex differentiation has occurred. At this time we will analyze the sex ratios observed in the diploid, triploid and gynogenetic groups. These ratios will provide preliminary information regarding the sex determination mechanisms of white sturgeon, and could be of commercial interest to caviar producers.

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MOLECULAR PHYLOGENY OF NORTH AMERICAN ACIPENSERIFORMES

DERIVED FROM RIBOSOMAL RNA GENE SEQUENCES

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Introduction

The order Acipenseriformes is an ancient group of fish identifiable from the Jurassic (Grande and Bemis, 1991). The group is characterized by a highly cartilaginous skeleton, heterocercal tails and a well developed rostrum with mouth inferior (Sokolov and Berdichevskii, 1989). At present, it consists of approximately twenty-four Recent species of sturgeon and two species of paddlefish. These fish are diadromous or strictly freshwater, with a North American and Eurasian distribution. All species investigated cytologically appear to be polyploid, possessing large numbers of chromosomes. These fish appear to be separated into two groups, one possessing about 120 chromosomes and the other approximately 240. The largest number of chromosomes actually counted is 258 (Birstein and Vasiliev, 1987; Blacklidge and Bidwell, 1993; Dingerkus and Howell, 1976; Fontana, 1994). Sturgeon and paddlefish have long life spans, can grow to become very large in size and, in general, take a long time to reach sexual maturity; females often require ten to twenty years before reproduction in nature (Lauder and Liem, 1983). Exploited for caviar and meat, their popularity as a food source in a number of countries has resulted in frequent over fishing. Recently, the population sizes of many acipenseriform species have decreased to the point where they are threatened or endangered. The decrease can be traced to three major factors: 1) the life histories of these fish; 2) the destruction of spawning habitat by pollution, dams or other human intervention; and 3) over fishing.

Information about the evolutionary relationships of the extant species of sturgeon and paddlefish may be useful in directing conservation efforts towards protecting a maximum diversity of Acipenseriform species. Because these relationships are not well understood, and since the "living fossil" status of the group makes them important for understanding vertebrate evolution in general (Birstein, 1993), a molecular phylogenetic study employing ribosomal RNA (rRNA) genes is being carried out in our laboratory. We have determined sequences from the nuclear 18S rRNA and mitochondrial 12S rRNA genes in order to examine the relationships between nine North American sturgeon and paddlefish taxa: *Polyodon spathula* (North American paddlefish), *Acipenser fulvescens* (lake sturgeon), *Acipenser brevirostrum* (shortnose sturgeon), *Acipenser transmontanus* (white sturgeon), *Acipenser medirostris* (green sturgeon), *Acipenser oxyrhynchus oxyrhynchus* (Atlantic sturgeon), *Acipenser oxyrhynchus desotoi* (Gulf sturgeon), *Scaphirhynchus platorynchus* (shovelnose sturgeon) and *Scaphirhynchus albus* (pallid sturgeon).

Ribosomal DNA (rDNA) has been widely used in phylogenetic studies to resolve the evolutionary relationships between organisms at various levels of taxonomic relationship (Spears et al., 1992; Kranz et al., 1995). The rRNA genes were chosen for the analysis of the Acipenseriformes for several reasons. The 18S rRNA gene produces the cytoplasmic small subunit ribosomal RNA, a molecule with highly conserved function and structure, normally consisting of 1700-1800 nucleotides. This gene contains conserved regions of primary structure that can be used to align sequences from different taxa and which can be exploited to construct "universal" primers for

amplification by the Polymerase Chain Reaction (PCR) and for DNA sequencing (Hillis and Dixon, 1991). It also contains variable regions that are phylogenetically informative and may be useful for determining relationships between species. The 18S rRNA gene is found universally in eukaryotes along with the 28S (nuclear large subunit) rRNA gene and the 5.8S rRNA gene in a single transcription unit (Figure 1). The ribosomal DNA transcription unit can be found on various chromosomes in different organisms as a tandem repeated sequence. There may be hundreds or even thousands of copies of the rDNA transcription unit in a particular species' individual cells (Appels et al., 1980), but there may also be as few as one copy (Yao and Gall, 1977). The number of copies found in the genomes of sturgeons is at present unknown. Despite the large number of rDNA repeats in an array, the process of concerted evolution is thought to rapidly homogenize all arrays of a particular repetitive sequence within an individual and a species (Arnheim et al., 1983). Such a homogenization process appears to have acted on the rRNA genes, whose tandem repeats form a type of repetitive sequence, in almost all species examined to date. Consequently, it is normally sufficient to sequence a single copy of the 18S rRNA gene in of a species for analysis in molecular phylogenetic studies. We have discovered, however, that this may not be true of the rRNA genes of the sturgeons. Finally, to aid in phylogenetic reconstruction, 18S rRNA gene sequences from several other fish are also available for comparison.

The second gene used in this study, the mitochondrial 12S rRNA gene, also codes for a small subunit ribosomal RNA, one which is restricted to the mitochondrion. The gene is smaller than the nuclear 18S rRNA, being 950 base pairs long. Because it is a mitochondrial gene, it is maternally inherited, and the equivalent of a single 12S rRNA gene sequence should normally be present in an individual, even if the organism is polyploid. In animals, mitochondrial DNA evolves more rapidly than nuclear genes, possibly due to decreased accuracy of the mitochondrial DNA polymerase and less efficient DNA repair mechanisms (Brown et al., 1979; Clayton et al., 1974). PCR primers have been designed which are complementary to transfer RNA gene sequences which flank the genetic region containing the mitochondrial control region and 12S rRNA gene sequences. These primers can be used to amplify the entire 12S rRNA gene from all the North American sturgeon and paddlefish. Although the data base of sequences of the 12S rRNA gene is limited, these other properties make the gene another good candidate to help clarify acipenseriform evolution.
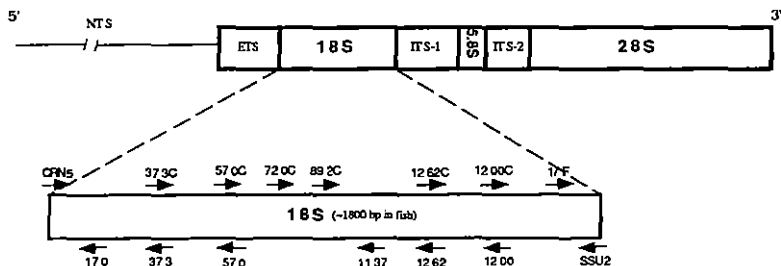
Figure 1. Diagram of the nuclear rDNA array of eukaryotes. The figure represents a single transcription unit of rDNA that is found in tandem repeats on various homologous and/or non-homologous chromosomes of an organism. (NTS = non-transcribed spacer; ETS = external transcribed spacer; ITS = internal transcribed spacer.) The detail of the small subunit (18S) rRNA gene shows the location of PCR primers (and their orientations) used in this study for amplification and sequencing of the gene.

Methods

Tissue samples were obtained from various researchers, fisheries units or from aquarium retailers. Samples included those of the nine sturgeon and paddlefish taxa listed previously, and of several additional species important for determining the position of the Acipenseriformes relative to other fish groups. These other species include *Protopterus annectens* (African lungfish), *Amia calva*

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(bowfin), *Lepisosteus osseus* (longnose gar), and three species of polypterids, *Polypterus senegalus*, *Polypterus delhezi*, and *Erpetoichthys calabaricus*. Tissue samples were in the form of muscle, blood, barbels or fin snips, with fin snips being the preferred type because they are the least intrusive to the animals. Genomic DNA was extracted from the tissues using a digestion with proteinase K in ABI lysis buffer followed by phenol-chloroform extraction and ethanol precipitation. DNA was resuspended in TE buffer and quantified using a spectrophotometer. PCR amplification (Saiki et al., 1985) of 18S rRNA or 12S rRNA genes from genomic DNA was carried out with primers designed in our laboratory (Figure 1). Two identical PCR reaction products were pooled for each gene to compensate for possible errors caused by the Taq polymerase that may occur during amplification. DNA sequencing of PCR products was performed either by the direct cycle sequencing (BRL) of the product, or by first cloning the product into a bacterial plasmid using the TA cloning protocol (Invitrogen) followed by cycle sequencing of the clones.

Entire 18S rRNA gene sequences (~1800 base pairs) were determined for *P. delhezi*, *P. senegalus*, *E. calabaricus*, *P. annectens*, *A. calva*, *L. osseus and Polyodon spathula*. Only partial sequences of the gene have been completed for the eight sturgeon species, because of the unexpected discovery of intraindividual variation. The 18S rRNA gene sequences were aligned with other fish sequences which had been completed previously in our laboratory or which were retrieved from the GENBANK database or from other publications. Alignment was carried out with the computer program ESEE (Cabot and Beckenbach, 1989), with the aid of a secondary structure of *P. spathula* small subunit rRNA that was constructed based on accepted secondary structures (Gutell, 1994). Partial 12S rRNA gene sequences were determined for the eight acipenseriform species as well as the three polypterid species, which were used as the outgroup. The partial sequence from the twelve species was aligned using ESEE. Phylogenetic analyses were conducted using MEGA (Kumar et al., 1993) for a neighbor joining analysis and PAUP (Swofford, 1990) for parsimony analyses.

Results

The eight sturgeon taxa were found to possess substantial intraindividual variation in the 18S rRNA gene. This was unexpected, and represents one of the first examples of widespread intraindividual heterogeneity of rRNA gene sequences in any eukaryote. As a result of this finding, it will be necessary to clone variants present in each species before sequences can be correctly determined for analysis. It is not clear whether intraindividual heterogeneity will have any effect on estimates of interspecific differentiation. For only one species, the white sturgeon *A. transmontanus*, have we cloned multiple sequences to date, and clones currently involve only the 5' half of the gene (~1200 base pairs). Ten clones have been isolated and sequenced. They represent six different sequences of four distinct types.

Even though all the species of acipenseriforms could not yet be compared using the 18S rRNA gene, the placement of the Acipenseriformes with respect to other orders of fish can be examined. The paddlefish was found to contain only a single 18S rRNA gene sequence. We also used the cloned sequences of the white sturgeon. These cloned sequences were aligned with the 5' segments of the 18S rRNA gene sequences from other fish species and a consensus neighbor-joining tree was constructed from the data using MEGA. This tree is shown in Figure 2. In addition, a consensus parsimony tree was constructed using the same data using the computer program PAUP for comparison (tree not shown). In both cases, *Styela* (a tunicate) was used as the outgroup. All the non-sturgeon species of fish mentioned above could be included in this study. These sequences could be obtained without cloning, because the species possess only one 18S rRNA gene sequence.

Partial mitochondrial 12S rRNA sequences (~250 base pairs) have been obtained and aligned from the acipenseriform species and three polypterid species. As mentioned previously, only one sequence was found to be present for this gene in all the species examined. This allowed a preliminary comparison of the sequences from all the North American sturgeon and paddlefish in an attempt to clarify species relationships within the Acipenseriformes. Shown in Figure 3 is the consensus neighbor-joining tree constructed using MEGA. PAUP was used to construct a consensus parsimony tree based on the same data for comparison. In these analyses, the polypterids were used as the outgroup.
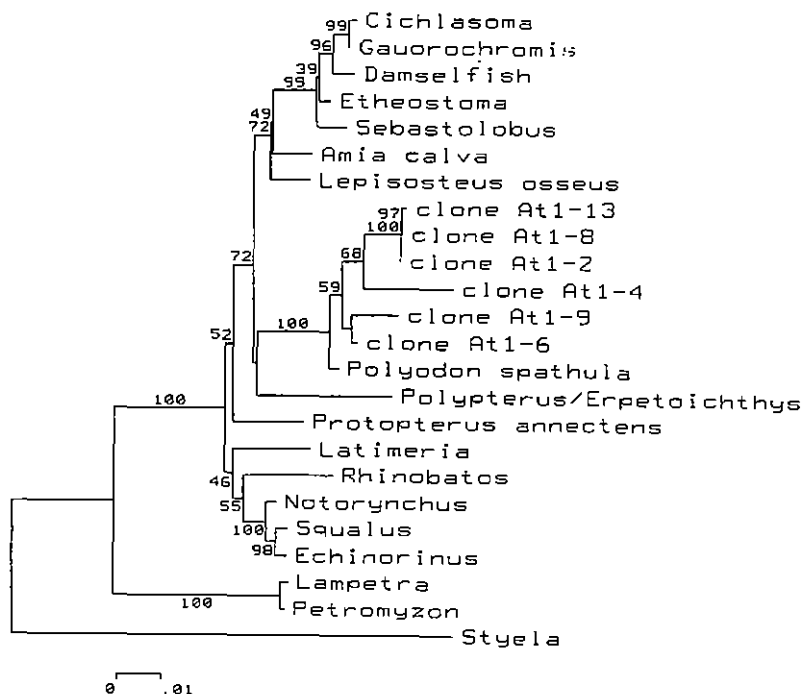
Cichlasoma 99
Gauorochromis 96
Damselfish 39 / 99
Etheostoma
Sebastolobus 49 / 72
Amia calva
Lepisosteus osseus
clone At1-13 97
clone At1-8 100
clone At1-2
clone At1-4 68 / 59
clone At1-9 100
clone At1-6
Polyodon spathula 72
Polypterus/Erpetoichthys 52
Protopterus annectens 100
Latimeria
Rhinobatos 46
Notorynchus 55
Squalus 100
Echinorinus 98
Lampetra
Petromyzon 100
Styela

0 — .01

Figure 2. Consensus neighbor-joining tree based on partial (~1200 bp) nuclear 18S rRNA gene sequence including *A. transmontanus* (At1) clones (1000 bootstraps). A parsimony analysis of the same data using PAUP produced a consensus of the four most parsimonious trees which shows the same topology except for the placement of *Latimeria*. Numbers on branches represent bootstrap values.

Discussion

In Figure 2, the consensus neighbor-joining tree demonstrates relationships between the major orders of fish based on ~1200 bases of the 18S rRNA gene sequences. In general, this tree agrees with relationships recently proposed based on morphological data. The lampreys are basal to the entire group, followed by the cartilaginous sharks. The African lungfish is placed outside the Actinopterygii (teleosts, bowfin, gar, Acipenseriforms and polypterids). The gar and bowfin are clustered with the teleosts, with the bowfin slightly closer to the teleosts than the gar. Important for the purposes of this paper, the North American paddlefish clusters with the *A. transmontanus* (At1) clones, as expected since the families Polyodontidae and Acipenseridae are grouped together within the Acipenseriformes.

Variability within *A. transmontanus* clones does not affect their relationship with other species, since the six clones cluster together, separated into four different sequence types. As mentioned, the presence of intraindividual heterogeneity of the 18S rRNA gene in sturgeons is unusual. The sequence of the rDNA region appears to be homogenized to a single sequence type in almost all organisms previously examined, presumably by the process of concerted evolution. One hypothesis to explain the apparent difference between sturgeons and other eukaryotes is that the
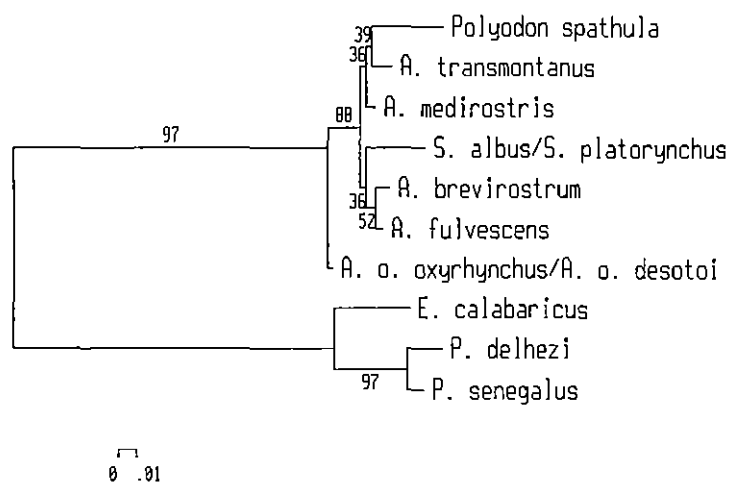
Figure 3. Consensus neighbor-joining tree based on partial (~250 bp) mitochondrial 12S rRNA gene sequence. In a parsimony analysis of the same data, a consensus of the nine most parsimonious trees shows the same basic topology but fails to resolve the branching order for *P. spathula*, *A. transmontanus*, *A. medirostris*, and the *Scaphirhynchus* species.

heterogeneity in these fish results from the high ploidy levels of sturgeon. One point that needs to be stressed here is that although ploidy level may be the cause of the variation, ploidy level does not necessarily always lead to intraindividual heterogeneity. The North American paddlefish is considered to be a tetraploid, according to results of flow cytometry and karyotypic studies (Blacklidge and Bidwell, 1993; Dingerkus and Howell, 1976), but it was not found to possess intraindividual variation of the 18S rRNA gene.

There are two major differences between the 18S rRNA gene tree and some current hypotheses about the evolutionary affinities of the Acipenseriformes. First, the polypterids were found to cluster (although weakly) with the Acipenseriformes, together with which they form a sister group to the clade which contains the teleosts, the bowfin and the gar. This branching pattern occurs in both neighbor-joining and parsimony analyses of the data. Our results support the view that the Polypteridae and the Acipenseriformes should be grouped together within the Chondrostei as part of the Actinopterygii. In other words, the polypterids do not belong in their own subclass. An additional interesting point is that identical 18S rRNA gene sequences were obtained for the three polypterids used in this study, so that the species level relationships could not be resolved with this gene. Second, analysis of the 18S rRNA gene does not result in a clustering of the coelacanth (*Latimeria chalumnae*) with the lungfish. Rather, *Latimeria* clusters with the sharks. However, in the parsimony tree, the coelacanth is shown branching off the line leading to the Actinopterygiians before the lungfish, and after the divergence of the Chondrichthyes. This latter tree agrees with most morphological based classifications that place the lungfishes and coelacanth together in the subclass Sarcopterygii. Conflicting results using the two reconstruction methods do not allow us to support or refute the current placement of *Latimeria*.

Figure 3 presents the consensus neighbor-joining tree based on partial mitochondrial 12S rRNA sequences of North American acipenseriforms and three polypterids. It is evident from this tree

that 12S rRNA gene sequences are sufficiently divergent to distinguish between the polypterid genera and species, which could not be separated using the larger sequences of the 18S rRNA gene. The tree should show even better resolution as additional sequence information from the entire 12S rRNA gene is added. As regards the relationships between sturgeon species illustrated here, the short branch lengths indicate that these species are very closely related. In fact, the two *Scaphirhynchus* species have identical nucleotide sequences for this portion of the gene. This is also true for the Atlantic and Gulf sturgeons (*A. o. oxyrhynchus* and *A. o. desotoi*). The grouping of some *Acipenser* species in this reconstruction appears to be related to their species ranges. The two Eastern American species, *A. fulvescens* and *A. brevirostrum*, cluster and the two Northern Pacific species, *A. transmontanus* and *A. medirostris*, are shown together as well. The Atlantic *A. oxyrhynchus* species is shown basal to the whole group. The relationships between the *Acipenser* species in this tree correlate well with those proposed in a cladogram published in the April 1995 issue of The Sturgeon Quarterly by Evgenii N. Artyukhin in his article "On Biogeography and Relationships Within the Genus *Acipenser*". Artyukhin's cladogram shows the same branching pattern, indicating the same relationships between these six species as shown in our tree. However, the placement of the genera *Scaphirhynchus* and *Polyodon* in the 12S rRNA gene tree is not that predicted by the analysis of morphological data. The *Scaphirhynchus* species are shown within the genus *Acipenser*. These two species are currently classified in two different subfamilies. However, the genus *Scaphirhynchus* is pleisiomorphic, possessing ancestral characters. This would be consistent with the location of these species within the *Acipenser* group as shown in our molecular analysis. *Polyodon*, which is considered a derived form of paddlefish by Grande and Bemis (1991), is shown in the 12S rRNA tree as a derived species within the Acipenseridae. More information is needed to resolve such apparent conflicts between our molecular data and morphological data.

Future work to clarify the relationships between the apparently closely related sturgeon and paddlefish species will concentrate on cloning and sequencing the 18S rRNA genes from the North American sturgeon species with intraindividual variation, completing the 12S rRNA gene sequences for the acipenseriform species as well as the polypterids, and for some other fish, and investigating other potentially more rapidly evolving genes for suitability for use in this phylogenetic study.

Acknowledgments

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Aquaculture and Disease

PROBING THE ENDOCRINE CONTROL OF STURGEON REPRODUCTION

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Sturgeon and paddlefish (Chondrostei) are perhaps the closest among living fish to the common ancestor of ray finned fish and tetrapods. The reproductive physiology and development of sturgeon exhibit many features common to amphibians and mammals, such as the primary structure of the gonadotropin-releasing hormone (Lescheid et al., 1995), the nucleotide and derived amino acid sequences in the vitellogenin gene (Bidwell and Carlson, 1995), and embryonic development (Dettlaf et al., 1993). Unlike teleosts, sturgeon have a hypothalamic-pituitary portal system controlling pituitary function like the tetrapods. Because of their unique place in vertebrate evolution and because so little is know about the function of their neuroendocrine systems, there is considerable interest to elucidate how these fish control reproduction and whether or not their neuroendocrine regulation more closely resembles tetrapods or teleosts.

Major interest in the neuroendocrine regulation of sturgeon has been generated by recent attempts to raise sturgeon in captivity for commercial aquaculture and to maintain stocks of threatened sturgeon species. Captive sturgeon females do not spontaneously spawn, necessitating the hormonal induction of ovulation and the surgical removal of eggs for fertilization. Ovarian development in captive sturgeon is long and variable, resulting in cultured broodstock of the same age being heterogenous with regard to sexual maturity. The level of sexual maturity cannot be recognized by external characteristics, requiring that the stage of maturity be determined by periodic ovarian biopsy. Because of the late sexual maturity, size and number of fish that must be monitored, the economic cost to the aquaculture industry is significant. For this reason, expansion of the sturgeon aquaculture will be dependent upon hormonal manipulation of reproduction.

The culture techniques that have been developed for commercial broodstock management are now being applied to save threatened and endangered sturgeon stocks, but similar problems concerning the control of reproduction in captivity also impact efforts directed at the conservation of sturgeon species. Because the numbers of captive individuals from these threatened populations are limited, it is equally important that hormonal manipulation of reproduction be used to insure ovulation, fertilization and identification of reproductively active fish held for breeding.

With the development of sturgeon culture, there are now fish available under controlled conditions to permit systematic studies on reproduction. Recently, we have made efforts in understanding the endocrine control of reproduction in white sturgeon (*Acipenser transmontanus*). As will be discussed, we have found that the neuroendocrine control of reproduction in this species seems to resemble the systems that have evolved in teleosts.

Reproduction in Cultured Sturgeon

By monitoring the plasma concentrations of hormones and ovarian development of over 500 females maintained at several cooperating aquaculture facilities, we have been able to follow the gonadal cycle in these fish. By 2 years of age, white sturgeon become sexually differentiated and the oocytes start meiosis. By 3-4 years of age, primary oocytes reach 200-300 μm diameter and are surrounded by a basal lamina and thecal layer. They remain in the refractory stage for a varied time (1-5 years) before the onset of the vitellogenic cycle. Three successive morphological changes characterize the onset of the first cycle: (1) proliferation of granulosa cells and differentiation of the inner follicular layer; (2) synthesis of the vitelline envelope, accompanied by the enlargement of the oocyte to 400-600 μm in diameter; and (3) deposition of the primordial yolk platelets in the peripheral cytoplasm. These three processes are followed by the vitellogenic growth of the oocyte reaching 3.5-4.0 mm in diameter at the completion of vitellogenesis (Doroshov et al., 1991). The increase in plasma vitellogenin concentration is evident only after the completion of the first two events. Concentration of vitellogenin in the circulation continues to increase towards the end of oocyte growth, and decreases before spawning (Linares-Casenave et al, 1994).

In captivity, the reproductive development in a large number of maturing females remains arrested at the previtellogenic stage. Even in those females that advance to the spawning, oocyte maturation and ovulation does not occur spontaneously, necessitating the hormonal induction of spawning events. Unlike females, cultured males mature more rapidly and do not present a significant practical problem in captive breeding.

Further development in the captive breeding of sturgeon requires that endocrine control of reproduction be understood sufficiently to develop endocrine and environmental treatments that will insure the early and synchronous onset of vitellogenesis, and non-invasive methods to assess reproductive development. Understanding the endocrine control of reproduction and gametogenesis of sturgeon will provide solutions to these three problems that are hampering the full potential of sturgeon breeding.

Sturgeon Gonadotropin

In teleosts, considerable evidence has developed supporting a dual gonadotropin (GTH) system controlling reproduction (Suzuki et al., 1988a, b, c; Swanson et al., 1991; Van Der Kraak et al., 1992; Okada et al., 1994). We have also identified two glycoprotein fractions from sturgeon pituitaries (Moberg et al., 1991) that have physiological characteristics (Moberg et al., 1995) suggesting that they may be functional analogs of the gonadotropin, GTH I and GTH II, found in salmonids (Kawauchi et al., 1989; Swanson et al., 1989). Because of this similarity, these two glycoproteins are designated stGTH I and stGTH II. Our preliminary studies suggest that stGTH I induces and maintains vitellogenesis and that stGTH II stimulates final ovarian maturation and spawning. During reproductive development there is a significant change in the pituitary content of the two stGTHs in both the male and female sturgeon (Moberg et al., 1995). In the vitellogenic female, the pituitary concentration of stGTH I is higher than stGTH II. Only at the end of the vitellogenic phase does the amount of stGTH II begin to increase reaching significantly higher levels during ovulation (Moberg et al, 1995). While both gonadotropins induce GVBD *in vitro*, stGTH II seems to be the most effective (Moberg et al., 1991). The evidence accumulated to date supports the assumption that these two GTHs have physiological actions similar to what has been reported in salmonids.

Hypothalamic Factors

Two forms of gonadotropin releasing hormones have been found in sturgeon brain, mammalian and chicken II GnRH (Sherwood et al., 1991; Lescheid et al., 1995). The major sturgeon GnRH is

similar in structure to mammalian GnRH (Sherwood et al., 1991) and a synthetic analog of mammalian GnRH, [D-Ala[6], Pro[9] N-Et]-mGnRH (GnRHa), has been found to be effective in inducing ovulation and spermiation in sturgeon (Doroshov and Lutes, 1984; Fuji et al., 1991; Gontcharov et al., 1991). We have found that a single injection of GnRHa will induce the secretion of stGTH I in mature males with only a marginal effect on stGTH II (Moberg et al., 1995). The magnitude of this response appears to be positively correlated to the plasma concentrations of testosterone existing prior to the injection of GnRHa (Moberg and Doroshov, 1992). While GnRHa will not induce stGTH secretion in previtellogenic females, it will induce the secretion of both stGTHs in females at spawning.

In several species of teleosts (Peter et al., 1991) dopamine acts as an endogenous inhibitor of GnRH-induced pituitary secretion of GTH, supporting the view that there is a dual hypothalamic neuroendocrine mechanism controlling GTH secretion with GnRH having a stimulatory effect on the synthesis and secretion of GTHs and dopamine inhibiting the secretion of the GTHs. No comparable evidence has been developed for this type of regulation in the Chondrostean fish. We treated mature male white sturgeon with dopamine (100mg/kg) in combination with GnRHa and found that dopamine effectively suppresses the ability of GnRHa to induce the secretion of both stGTHs. In contrast, the dopamine antagonist pimozide potentiates the ability of GnRHa to stimulate the secretion of GTHs in spermiating sturgeon. These data strongly suggest that, unlike mammals (Swartz and Moberg, 1986), sturgeon have a dual hypothalamic control of GTH secretion similar to some teleosts.

Influence of Steroids on Gonadotropin Secretion

Several studies on teleosts have demonstrated that gonadal steroids stimulate the pituitary accumulation of GTHs (Crim and Peter, 1978, 1979; Crim et al., 1981; Magri et al., 1985; Dufour et al., 1983). As discussed, we have observed a positive correlation between plasma concentrations of testosterone and the amount of stGTHs secreted in response to the administration exogenous GnRHa, suggesting that gonadal steroids might stimulate the accumulation of pituitary stGTHs. To explore this possibility, we implanted 18-month old sexually undifferentiated sturgeon with testosterone (Pavlick, et al., 1995) and found that the pituitary concentrations of stGTH I and stGTH II were significantly elevated following the implantation. However, there was no significant effect of testosterone treatment on the plasma concentrations of either stGTH. When the testosterone treated fish were injected with GnRHa, there was no increase in the plasma concentrations of either stGTH. Thus, while exogenous testosterone induced the accumulation of stGTHs in the pituitaries of juvenile white sturgeon, GnRHa was ineffective in stimulating the secretion of these stGTHs.

In continuing studies, 24 month-old sturgeon were implanted with testosterone and the fish were monitored over several months. After 90 days of exposure to testosterone, the pituitary concentration of both stGTHs were elevated, but even after prolonged treatment with testosterone there was no significant change in the circulating levels of the stGTHs. Again, even with elevated pituitary concentrations of the two stGTHs, exogenous GnRHa was incapable of inducing secretion of the stGTHs, suggesting that these immature fish may have nonfunctional GnRH receptors and/or there is strong negative feedback of testosterone or other endogenous inhibitors in these prepubertal fish.

Oocyte Maturation

The developmental biology of the sturgeon oocyte has been described by Dettlaf and co-workers (Dettlaf et al., 1993). However, the role of gonadal steroids in regulation of final ovarian maturation remains to be elucidated. In white sturgeon, the oocyte maturation is readily induced in vitro by C-21 steroids, with 17α-Hydroxyprogesterone and 17α, 20β-Dihydroxy-4-pregnen-3-one

(17α20β-DP) being the most potent (Lutes, 1985). Recently we have observed that domestic white sturgeon females consistently exhibit significant increases in plasma concentrations of 17α20β-DP at ovulation temporarily correlated with the increase of plasma stGTH II. Similar data have been obtained following the induction of spawning in wild-caught white sturgeon (Lutes et al., 1984) and in Atlantic sturgeon caught on their spawning grounds in the Hudson River (Van Eenennaam et al., In Press). In salmonids, 17α20β-DP has been already identified as the native oocyte maturation-inducing hormone (Young et al., 1986). While the identity of the putative maturation-inducing hormone has not been established for sturgeon, the other similarities between salmon and sturgeon may with respect to the endocrine control of reproduction, strongly suggest that as in the case of gonadotropin regulation, oocyte maturation in sturgeon may be similar to salmonids.

Environmental Factors

While the amount of information concerning the effect of environmental factors on GTH secretion in sturgeon is limited, it seems reasonable that season and water temperature would have a significant modulatory effect on these seasonal breeders. During the breeding of domestic sturgeon, we have observed poor spawning performance and egg quality which we have attributed to suboptimal water temperature as reported by Dettlaf et al. (1993). We examined the effect of water temperature on the endocrine events occurring during spawning induction. Females in the seasonal temperature treatment (rising from 10° to 15° C) exhibited a gradual decrease in plasma estrogen levels, but maintained high plasma levels of testosterone until hormonally induced ovulations. After the administration of GnRHa and carp pituitary extracts, these fish exhibited a pronounced ovulatory surge of stGTH II. Fish in the other two treatments (constant 15° and 19° C) exhibited a rapid decline in both plasma testosterone and estrogen, with concomitant arrest of germinal vesicle migration (Webb et al., 1994). Following GnRHa administration, stGTH II secretion and ovulatory response were significantly reduced compared to ripe females maintained in the cooler water. These data suggest that water temperature may have a significant modulating influence on the regulation of stGTH secretion and the secretion of sex steroids plays an important role during the postvitellogenic phase of ovarian development.

In preliminary studies examining the effect of season on GTH regulation, we found that mature males treated with GnRHa in August secrete little stGTHs, while in December (just prior to spawning season) and in April (during spawning season) significant amounts stGTHs are secreted in response to the exogenous peptide (Moberg et al., 1995). These preliminary data indicate that season may be an important modulator of stGTH secretion.

These data support our view that season and water temperature modulate the secretion of sturgeon GTHs. Consistent with this view is our observations of sturgeon spawning at commercial aquaculture farms which indicate that both seasonal photoperiod and water temperature are important environmental cues for sturgeon reproduction.

Summary

From the data obtained to date, we can make several assumptions about the control of GTH secretion in sturgeon. Like salmonids, sturgeon appear to have two gonadotropin controlling the reproductive cycle and gametogenesis. GnRHa will stimulate stGTH secretion in mature males and will induce spawning in mature females, but will not stimulate stGTH secretion in pre-vitellogenic females. Dopamine inhibits stGTH secretion in mature male sturgeon. The dopamine antagonist pimozide potentiates stGTH secretion in GnRHa-treated ripe males. Testosterone elevates the pituitary concentration of stGTH in immature sturgeon, but GnRHa will not stimulate the release of stGTHs in these fish. There is a seasonal influence on the responsiveness of the gonadotrope to GnRHa in males. Likewise, seasonal temperature influences final ovarian maturation in females. While sturgeon exhibit many of the ancestral reproductive and developmental characteristics of

tetrapods, the neuroendocrine control of reproduction of sturgeon seems most closely aligned with our understanding of teleosts.

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WHITE STURGEON CULTURE AT SIERRA AQUAFARMS, INC.

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Sierra AquaFarms, Inc. (SAF) is an intensive, partially recirculated White Sturgeon (Acipenser transmontanus) farm located in Elverta, California, a small community approximately 10 miles north of Sacramento. The company was originated in 1983 with the specific intent of developing methods for the culture of white sturgeon and the successful marketing of the finished product. SAF started out as a small research and development facility at another location. This facility was used to determine the suitability of white sturgeon as a culture animal. At this time white sturgeon culture in this country was in its infancy with the first successful artificial spawning accomplished by team of researchers led by Dr. Serge Doroshov at the University of California, Davis in 1979.

Utilizing the techniques developed by Dr. Doroshov, SAF began spawning fish in 1984 and looking at basic culture questions regarding feed types, stocking densities, disease considerations, and growth rates. Initial indications were that white sturgeon had culture potential. Despite the fact that many questions remained unanswered, the financial backers of the operation had sufficient confidence in the culture characteristics and marketability of the fish that in 1985 the decision was made to build a commercial size facility.

Four major site selection criteria were considered when deciding on the location for the commercial facility. The first criteria was to be geographically close to the migrating wild broodstock. At that time, wild gametes were the only source for fry production. Adult white sturgeon migrated annually up the Sacramento River and were a viable source of broodstock.

The second criteria was to be within close proximity to the expertise available at University of California, Davis. At that time research was underway on reproduction, disease, nutrition, and physiology. These resources have proven to be invaluable over the course of our commercial development.

In order to balance the needs of fish production with those of marketing and distribution, we felt that it was important to locate near good highways and an airport for product distribution and for easy access and egress for supplies and personnel.

The fourth criteria was an adequate water supply of appropriate water quality that would allow us to meet our production goals. This consideration proved the most difficult to satisfy. For the quantities of fish that we wanted to produce we would need water quantities in the order of tens of thousands of gallons per minute. If we simply intensified using pure oxygen, we could reduce

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this water requirement to thousands of gallons per minute. Additional technology could further reduce this water demand.

In an attempt to satisfy these four site selection criteria, it became apparent that we would need to utilize some form of recycle technology. Our goal was then to design and operate a system that would use approximately 3,800 liters per minute of pumped ground water and produce approximately 450,000 kilograms per year of white sturgeon. We purchased 21 acres of farmland and engaged a Scandinavian consulting firm in 1985 to collaborate on the design of our first building.

This building is approximately 2600 m^2 and houses a non-recirculated hatchery and a partially recirculated fingerling sizing area. We use pure oxygen injection throughout the facility but have chosen not to recirculate water in the hatchery due to the sensitive nature of the sturgeon fry and smaller fingerlings to water quality parameters.

The hatchery consists of 116 fiberglass tanks that are 0.9 x 3.7 x 0.5 meters deep. These tanks are built on two levels for space considerations. We chose this tank design because we wanted a long residence time of the feed in the tank so that the fry /small fingerlings would have a chance to ingest the feed before the feed goes down the drain. This feeding strategy was implemented due to our incomplete knowledge at that time of the nutritional requirements and the lack of aggressive behavior of the fish to the commercial diets available. The tanks worked very well in this respect but had one large disadvantage in that uneaten food and feces would tend to stay in the tank. We developed an automatic cleaning system that would periodically sweep the sides and bottom of the tank clean and push the feces/feed to the screened outlet. This outlet screen also has a set of brushes rotating at 1 rpm to eliminate any biofouling from clogging the screens. This system reduced our personnel requirements by 90% and increase fry/fingerling survival dramatically.

Control and monitoring systems in the hatchery are relatively simplistic. Systems include flow meters/alarms, feeder time delay relays, and dissolved oxygen meters. We inject pure oxygen in the hatchery via an Air Liquide oxygen cone. This system is set up as a two step control and allows us to maintain our oxygen levels at $+\backslash-0.5$ ppm of our target dissolved oxygen level.

When the farm was first designed, reliance on wild broodstock for progeny was the only choice. Domestic male and female broodstock are now available. Males generally range in weight from 18 to 45 kilograms and are a minimum of three years old. Females range from 30 to 100 kilograms in weight and have an average time to maturation of 7 to 8 years.

Water temperature at SAF ranges from 20 to 23 degrees Centigrade. This appears to be an optimal growing temperature but too high of a temperature for successful spawning. Mature males will successfully spermiate at these temperatures with the injection of spawning agents such as Common Carp Pituitary (CCP) or LHRH$_a$. Females require a period of cooler temperatures, preferably below 15 degrees Centigrade for several months, to complete their maturation process. For this reason, SAF currently moves our female broodstock to a cold water source at another location.

Selected females are biopsied by making a small ventral incision and aspirating about 50 oocytes. These oocytes are assayed for their suitability for spawning using a progesterone assay procedure. If the assay goes well, ovulation is induced by injection of CCP or LHRH$_a$. If all goes well, 24 to 36 hours after injection the female will ovulate. At this point the fish is transported by stretcher to an area where the surgery is to occur. A hose is inserted in the mouth of the fish to allow irrigation of the gills with oxygenated water. An incision approximately 12 centimeters in length is made ventrally in order to manually remove the eggs. After the eggs are remove, the female is sewn back up and returned to a tank.

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The eggs are then fertilized with previously collected sperm. The eggs exude a sticky coating when fertilized. To eliminate this problem, the eggs are gently mixed with silt to deadhere them. The eggs are then placed in MacDonald incubation jars for 7-10 days at which point hatching occurs.

Newly hatched larvae are self sufficient for 7-10 days at which point external feeding begins. Fry are started out on powdered feeds administered as a slurry and progress to crumbles and ultimately pellets. At the end of the first year our average selected fish are approximately 200-300 grams in weight.

At this stage the fish are transferred into our fingerling sizing system. This system consists of approximately 1000 m^3 of tanks and utilizes recycle technology to treat the water. Water in this portion of the system is received from the hatchery, where it has already been utilized once, and is then recycled another 10 times in this system. This fingerling sizing system consists of 56 tanks measuring 2 x 2 x 1.2 meters and 20 tanks measuring 6 x 6 x 1.2 meters. The water in these tanks is exchanged one time per hour. After the water exits a given culture tank, it is degassed of CO_2 via degassing towers and atmospheric oxygen is added via a counterflow of air. The water then enters screen filters which remove suspended solids down to 180 microns in diameter. The sludge is then separated by these filters and exits the system while the comparatively clean water is pumped into oxygen cones for pure oxygen injection, through pressure regulating valves, and back to the culture tanks. Concurrently another loop exists where the water exiting the screen filters is pumped to fluidized sand beds where nitrification occurs converting NH_3 to NO_3.

Because of the degree of recirculation that is used in this room, the automatic control and monitoring capabilities are more sophisticated. Control capabilities include oxygen injection rates, pressure regulation, pump speeds and pump on/off conditions. This system is monitored for 22 parameters including tank water levels, dissolved oxygen levels, water flows, air flows, and pressure alarms. Total water flow rates in this system are approximately 19,000 liters per minute.

Feeding is done automatically 20 hours per day at 20 minute intervals. The fish are fed extruded sinking pellets ranging in size from 1.5 to 7mm. Currently we are using a diet that is 43% protein and 14% fat. Fish are held in this system during the second year of their life until they reach a size of approximately 1.7 kilograms.

In 1990 we expanded our system into a second building approximately 3000 m^2 in size. This allowed us to have another 5500 m^3 of production tanks. Our fish typically enter this system when they are approximately 1.7 Kgs in weight and then stay in these tanks until they reach a marketable weight of 7 - 9 Kgs. This entire growth cycle takes about 4 years. When designing this building we were able to rely on the experience base we had acquired running the first building for 4 years. We were able to modify the design to create a more effective and efficient growing environment.

There are 13 tanks in this building. Each tank is 13 x 13 x 3 meters in size. Water flow exits the center of these tanks and flows directly to our fluidized sand beds for nitrification. These fluidized beds are considerably larger than the beds that we have in the other building. These beds measure 3 meters in diameter and 5 meters high. This increase in the filter/feed rate ratio allows us to reuse the water in this building to a higher degree than in the previous building. Water passes from the beds to a stripping tower for CO2 removal and then to an atmospheric pressure pure oxygenation column for reoxygenation. The water then returns to the culture tank. We then have a separate loop that is taking solids and water from the bottom of the tank and sending it to a set of screen filters where the solids are filtered out and the clean water is distributed back to the fluidized sand beds. Total flow rates in this building are 136,000 lpm.

Oxygen control on this system is a seven step control rather than the 2 step control that is found in our earlier systems. This seven step control is a more efficient method for injecting oxygen. We could justify the extra expense for this type of oxygen control based on the increase biomass in this portion of the system. Oxygen is monitored in the tank, in the discharge of the tank, and on the top of each our fluidized sand beds.

Target densities are around 60 Kg/m³. Combined building biomass capacities are approximately 400,000 Kgs. Feeding is done by a feeding robot that runs around the building on a rail. We currently feed 7mm and 10.5 mm pellets to the fish in this system every 20 to 25 minutes. The robot accurately meters the type and amount of feed we want into the tank in whatever schedule that we decide upon. It has an infrared communications link to our computers and downloads information every feeding cycle.

The entire system is run by a programmable logic controller otherwise known as a PLC. This is an industrial process control that takes care of the system for us and is a step up from the basic electrical relay system we are using in the building that we designed earlier. This PLC monitors and controls oxygen, tank levels, valve conditions, water temperatures, pump operations, etc.. It also monitors alarm conditions and either takes care of the situation itself of decides to wake one of us up so that we can deal with the problem.

The farm complex includes two employee residences so that 24 hour coverage is maintained. Alarm systems are wired into both residences as well as to a central alarm company that will start calling/paging personnel if a problem arises. Rapid response times are critical in an operation such as this when the potential downside of more than 400,000 kgs of fish expiring in hours is considered.

The PLC has a communications link with a personal computer (PC) that graphically shows us what is happening in the system at any given time. The PC uses a generic process control program that was customized to our needs. The PC not only gives us current conditions, but allows us to archive data that trends parameters such as temperatures, dissolved oxygen levels, feeding profiles, condition reports, etc.. We can display screens for instance that will show us dissolved oxygen readings for the past hours or weeks so that we can see how the system is interacting. By monitoring trending of parameters such as this we can, on many occasions, observe the progression of conditions in the system that can be corrected before they become a serious production issue.

Once the fish have completed their growing cycle, that is attained a swimming weight of 7 - 9 kilograms, they are transferred to processing staging tanks. These tanks are supplied with pure well water and serve two purposes. The first purpose is to give our sales and marketing staff an understanding of how much fish is available to sell over the next several weeks. The second purpose is to allow the fish to purge any flavor conditions that have been acquired during the 4 year growth cycle in the recycle water.

Sierra AquaFarms has full fresh processing capabilities on site. Final form of our fresh product ranges from gutted to filleted. We also have a subcontractor smoke our product in hot and cold smoked forms that are then vacuum packaged and frozen. We are processing and shipping our product six days a week which assures our customers a high quality product available on a schedule that meets their demands.

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SPAWNING AND REPRODUCTIVE PERFORMANCE OF

DOMESTIC WHITE STURGEON (*ACIPENSER TRANSMONTANUS*)

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Introduction

A collaborative University-Industry White Sturgeon Broodstock Development Program was established in California eight years ago to obtain information on sexual maturation of captive sturgeon and to help the industry in developing broodstock reproductive management. Currently used broodfish originated from wild parents caught in the Sacramento and Columbia Rivers. They are raised in tanks under natural photoperiod, temperatures 16-20° C and are fed salmonid diets.

The sturgeon industry initially relied on seedstock obtained by spawning wild-caught fish. Aquaculturists began rearing broodstock in the early 1980's and today there are increasing numbers of ripe fish available for spawning each year. Intensive culture has accelerated growth and sexual maturation of white sturgeon compared to the wild fish maturing for the first time at an age 12-20 years. Practically all cultured males reach sexual maturity at age 4 years and spawn annually. Females exhibit variable rates of maturation, but the majority of fish reach first sexual maturity at age 7- 10 years.

Sturgeon females do not ovulate spontaneously in captivity although males spermiate occasionally. Two pituitary gonadotropins are involved in regulation of gonadal development (stGTH I) and spawning (stGTH II) of white sturgeon. Before spawning, concentration of GTH II in the pituitary increases significantly, but its release in circulation occurs only after hormonal injection to induce ovulation (Moberg et al., 1995).

To induce ovulation or spermiation, fish are treated with either gonadotropin releasing hormone analog GnRHa ([D-Ala[6], Pro[9] NEt] GnRH) or/and common carp pituitary extracts (CPE). However, treatment is effective only if the female has reached the responsive stage of final ovarian maturation, which is manifested by the advanced stage of germinal vesicle migration and the oocyte response with germinal vesicle breakdown to a maturation inducing hormone.

The life cycle of white sturgeon is currently closed in captivity, and domestic offspring are now being used for commercial grow out. However, broodstock management remains a difficult task, due to the longevity of the sturgeon reproductive cycle and complexity of spawning procedures.

Estimation of Spawning Time

Although wild sturgeon of San Francisco Bay are spring breeders (Kohlhorst, 1976; Doroshov and Lutes, 1984), captive females reach a preovulatory state during an extended time

(February-September) depending on rearing temperature and endogenous cycle of individual fish. To estimate the spawning season on different farms, eggs from ripe females are sampled in early winter (December-January). Each fish is weighed, placed on a hooded stretcher ventral side up, with a flow of freshwater maintained across the gills. Ovarian follicles are sampled by abdominal catheterization (Conte et al., 1988) and kept in sturgeon Ringer solution (Dettlaff et al., 1993). Thirty follicles are boiled and fixed in 10% buffered formalin for measurement by image analysis of the polarization index (PI, a ratio of the distance of the germinal vesicle from the animal pole to the oocyte animal-vegetal axis diameter) which typically ranges from 0.20 - 0.35 at this time. Under optimal temperature conditions (generally, between 10° and 14°C) the rate of GV migration is relatively constant, allowing for estimation of the expected spawning month, when the oocyte PI reaches a value ≤ 0.10.

A second ovarian biopsy is collected at the expected spawning month of individual females to verify the physiologically responsive stage of oocyte development. The *in vitro* oocyte maturation assays are conducted in Ringer solution at 16°C for 16 hours. Fifteen follicles with two replicates are incubated in control and with $5\mu g/ml$ progesterone (4-pregnene-3,20-dione, Sigma Co.). After incubation, the eggs are boiled, chilled on wet ice, stored in 10% buffered formalin, bisected and examined for GVBD. Females that exhibit a 70-100% GVBD response are spawned in 2-4 weeks after biopsy.

Spawning Procedures

Females are injected (IM) with a priming dose of ovulation-inducing hormone (10% of the total dose), and a resolving dose 12 hours later (the hormonal treatments are described below). Ovulation is expected 20-24 hours after the second injection at the holding temperature 15-16°C. Females are anesthetized in 100 ppm MS-222 for 15 minutes, and their eggs are removed by aseptic caesarian surgery (10-12 cm incision). Anesthesia is monitored during surgery lasting 40-60 minutes by alternating water supply to the gills between fresh water and 50 ppm MS-222 solution. The incision is closed by internal and external stitches (PDS suture, Ethicon). Fish are injected with antibiotics (oxytetracycline, 5mg/kg). Primary healing of the incision occurs within 1-2 months and complete healing in 4-5 months.

Eggs are inseminated *in vitro*, de-adhered and incubated in jars (Conte et al., 1988). Post-spawned females are held in a separate recovery tank, supplied with clean, flow-through well water. Spent females start vitellogenesis during the fall of the same year and can be spawned again in 2 years.

Data collected from spawned females included: estimation of the percent of eggs released into the body cavity at caesarian section (% ovulation); latency time (hours after the second injection to the time when eggs were first observed in the spawning tank); total number of eggs collected determined volumetrically, egg diameter measured by image analysis; fertilization success measured as a proportion of cleaving eggs at 5-7 hours after fertilization from a random sample of 200 eggs; and hatching success determined as a percent of hatched larvae to total number of eggs collected.

Males are selected by external appearance (full abdomen), held at temperatures 12-14°C, and are induced to spermiate with 1.5 mg/kg CPE. Water temperature is raised to 15-16°C at the time of injection and milt is collected by catheterization 24 hours later. The males are returned to the holding temperature if they are intended for repeated spermiation. They can be induced to spermiate 3-4 times at biweekly intervals.

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Spawning Results

Increasing numbers of domestic females have been available for spawning induction during the past 4 years (Table 1). The body size and age of females spawned has been similar and individual females ranged from 17-80 kg in body weight and were 6-15 years old. Latency time, mean egg diameter and number of eggs removed were also similar, but fertility and hatching success improved significantly over time. Ovulatory response to hormonal injections increased from 43% in 1992 to 76% in 1995.

The number of domestic females induced to spawn increased from 9 females in 1992 to 29 in 1995, while the hatchery spawning of wild caught females decreased from 21 to 1. Improved management of broodstocks and induced spawning have led to an increase in the number of F_2 offspring from 131,000 during 1992 to 1,667,000 in 1995.

Almost all spawned females exhibited caesarian incision healing, although a small number of fish were lost after first spawning. Two factors appear to be important in reducing post-surgery mortalities. The first is careful control of anesthesia during the surgical procedures. Secondly, it appears critical to have a dedicated recovery tank for post-spawned females.

Table 1. Spawning performance of domestic females ovulated in 1992-1995. Data are means and standard errors.

Year (N=)	Body Weight (kg)	Age (yrs)	Latency (hr)	Egg Diameter (mm)	Eggs Removed (#)	Success Fert. (%)	Success Hatch (%)
1992 (n=9)	33.6 2.9	10 0.4	22.9 2.5	3.39 0.18	104,712 10,866	33.3 10.2	13.9 6.2
1993 (n=18)	36.9 2.9	10 0.2	21.9 1.4	3.52 0.16	126,250 14,756	50.0 8.0	30.4 7.3
1994 (n=16)	40.2 3.4	10 0.5	22.6 0.8	3.44 0.18	128,738 12,070	74.9 4.7	31.7 6.4
1995 (n=29)	36.3 2.7	10 0.8	21.4 1.3	3.50 0.18	115,515 8,959	73.5 5.3	49.1 6.3

Hormonal Injection Regimes

Previously, wild-caught white sturgeon females were induced to ovulate on farms by administration of CPE which provided high rates of ovulation but variable fertilization and hatching success (Doroshov and Lutes, 1984). A greater number of fish available in 1995 allowed us to conduct preliminary evaluation of different hormonal treatments for the induction of ovulation. Females were given one of four different injection regimes (total dose): GnRHa (21-40 μg/kg), GnRHa (11μg/kg) + pimozide (10mg/kg), GnRHa (10μg/kg) + CPE (4.5mg/kg), or CPE (4.5mg/kg) only. Application of the mammalian GnRHa, alone or in combination with antidopamine pimozide, yielded higher hatchability compared to treatments with CPE, or CPE in combination with a priming dose of GnRHa (Figure 1). While ovulatory response and fertilization success were similar in different treatments, the hatchability was significantly lower in the CPE treatment (P<0.05). Data suggest that CPE, being a strong inducer of ovulation in sturgeon at the dose applied, may negatively affect egg quality.
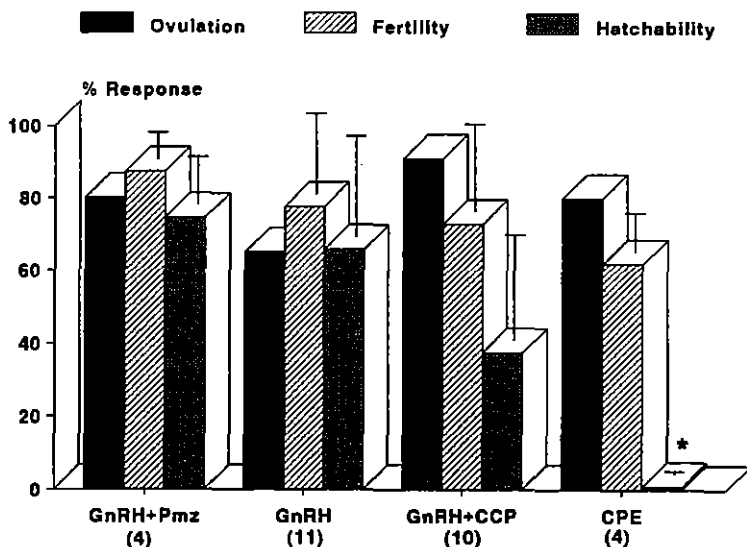
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Figure 1. Ovulation response, egg fertilization and hatching success in four different hormonal treatments (pooled females spawned at UCD and collaborating farms). Females that did not release any eggs, or those with less than 50% ovulation (estimated during collection of eggs) were considered non-responsive. An asterisk denotes significantly different means (P < 0.05). Data (mean ± SD) were processed by the analysis of variance with arcsine-transformed proportions.

Performance of Iteroparous Domestic Female Sturgeon

Six females were repeatedly spawned a second time and four females a third time during the period 1993-1996 (Table 2). All iteroparous females exhibited regular biennial intervals between consecutive spawnings on odd or even years. The exception was a single female that spawned a second time after a 3 year interval. There were significant increases in body weight, egg diameter and number of ovulated eggs obtained by cesarian surgery between each consecutive spawning (Table 2). Number of eggs collected may not accurately reflect the trends in individual fecundity, but suggests a 65 percent increase in production of eggs at third spawning. A small but highly significant increase in egg diameter extrapolates into a 17% gain in egg volume between first and second spawning and 27% between the first and third spawning.

Table 2. Reproductive performance of iteroparous females. Data are mean ± s.e.m. Superscripts denote significant difference between means (P < 0.05, paired T-test).

	Body Weight (kg)	Egg Diameter (mm)	Eggs Collected (th.)
	First to Second Spawning		
	(n=10)	(n=10)	(n=6)*
1st	33 ± 4^A	3.36 ± 0.06^A	102 ± 9^A
2nd	40 ± 4^B	3.54 ± 0.03^B	142 ± 17^B
% increase	21	5	39
	First to Third Spawning		
	(n=4)	(n=4)	(n=2)*
1st	37 ± 4^A	3.36 ± 0.08^A	113 ± 1^A
2nd	44 ± 5^B	3.53 ± 0.06^B	160 ± 5^B
3rd	51 ± 7^C	3.64 ± 0.03^C	186 ± 16^B
% increase 1-3:	38	8	65

* some data were deleted due to incomplete ovulation.

Discussion

The California sturgeon industry now relies entirely on spawning domestic broodfish. All techniques and methodologies to identify ripe females are now being transferred to the industry. During the next 2-3 years sturgeon breeders may become self sufficient in selecting and spawning white sturgeon broodstock.

The most critical aspects of broodstock care is the ability to maintain ripe fish at water temperatures ≤ 15° C during the spawning season. Spawning performance on the commercial farms was considerably improved in 1995 by holding gravid females at constant low temperatures (11-13°C), confirming observations of Kazanskii and Molodtsov (1973) with Russian sturgeon and Williot et al. (1991) with Siberian sturgeon.

During previous years the industry had some problems with caesarian incision healing and post-spawning mortalities, but with improved methods of using internal and external suture, the incision healing and survival of fish improved significantly. However, there are still some post-spawning mortalities. It appears that the best way to minimize these mortalities is to have a dedicated recovery tank that is used for maintaining post-surgery females for at least two months. This tank should be observed daily, have good water flow and water quality, feeding should start in about 3 days after surgery at approximately 0.3% body weight per day, and the feeding activity should be monitored.

Historically, ovulation in wild-caught white sturgeon was induced by administration of CPE (Doroshov and Lutes, 1984; Conte et al., 1988). However, these preliminary studies suggest that CPE, being a potent ovulatory stimulant for sturgeon, may negatively affect oocyte maturation (nuclear and cytoplasmic), resulting in abnormal gastrulation and variable hatching

success. In contrast, the administration of GnRHa, particularly in combination with the dopamine antagonist pimozide, significantly improved survival from fertilization to hatching. Considering the small number of conducted spawning trials and variations in environmental conditions on different farms, this conclusion is preliminary, and further research for optimization of hormonal spawning induction should be conducted.

Observations on repeatedly spawned, individual females, provide the first evidence of a biennial breeding interval in domestic broodstock of white sturgeon. Iteroparous females may have significant value for breeding, because they produce a greater number of larger eggs and have a more predictable endogenous ovarian cycle, requiring minimum efforts in monitoring ovarian development. In addition, they can be used in a selective breeding program based on progeny evaluation. Their disadvantages are large body size, and difficulty in handling and spawning.

The eight years of the collaborative Broodstock Development Program has yielded new information on the reproductive development of white sturgeon and resulted in establishing a viable commercial sturgeon industry.

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COMPARATIVE ANALYSIS OF CHEMOSENSORY SYSTEMS

IN FEEDING BEHAVIOR OF STURGEONS

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Introduction

In most fish species the main source of information about the presence, localization and qualitative features of food organisms is vision. In sturgeons visual reception is not functionally well developed and allows only the distinction of sharp variations in illumination and large moving contrasting objects. Sturgeons. At such fishes, having functionally extremely poorly advanced vision, to which concern many bottom, deep-water and cave fishes and fishes with night peak of activity the large development received the chemosensory systems, first of all olfaction and taste (Kleerekoper, 1969). As was shown (Pevzner, 1981; Pjatkina, 1991; Devitsina, Kazhlaev, 1995, etc.) sturgeons have a well-developed olfactory organ as well as both external and intraoral taste buds.

Olfaction is the main distant sense enabling sturgeon to receive the information concerning the food presence (Pavlov et al., 1970) or presence of ripe male and female are ready for spawning (Kasumyan, 1993). Extraoral and intraoral taste systems play very significant role in latest stages of feeding behavior in which sturgeon as other fish estimate the palatability of food items.

During last less than 10 years it was performed intensive investigations of olfactory, extraoral and intraoral taste sensitivity different acipenserid species to many natural and art chemical stimuli - water extract of living food organisms and both artificial feeds and their components, free amino acids and classical taste substances (Kasumyan, 1992, 1994, 1995; Kasumyan et al., 1992, 1993, 1995; Kasumyan and Kazhlaev, 1993; Kasumyan and, Taufik, 1994) . In all these studies it was used original methods based on fish feeding behavioral responses. In present paper it will be compare the behavioral responses and functional peculiarities of olfaction, extraoral and intraoral taste systems in different species belonging to family *Acipenseridae*.

Materials and methods

The subject of our investigations were Russian sturgeon, *Acipenser gueldenstaedti*, Siberian sturgeon, *A. baeri*, stellate sturgeon, *A stellatus*, green sturgeon, *A.medirostris* and hausen, *Huso huso*. It was used the hatchery fish of several age-groups, beginning with larvae at the stage of mixed feeding and ending with juveniles at the age of yearling. For the control of food

consumption motivation (feeling of hunger) fish were feed with living chironomid larvae only once a day after finishing the experiments.

Olfaction. The individuals (6-10 specimens) were permanently kept in special aquaria or basins with closed water closed circulation and a biological water-cleaning filter. From the biofilter the water went into parallel compartments of the aquarium constantly and at equal speed. In the course of the experiment the supply of clean water in one of the compartments was replaced by the test solution for 2-3 minutes.

The intensity of the fish behavioral responses was estimated on a 5-point scale which took into account the degree of disturbance of the initial organization of the fish in the aquarium, their ability to localize the compartment with the test solution, and their display of special behavioral patterns. The procedure of the experiments, evaluation of the intensity of the observed behavioral responses, deprivation of the sense of smell and the features of keeping the test fishes are detailed in (Kasumayn and Kazhlaev, 1993; Kasumyan and Taufik, 1993).

Taste. About 10-15 minutes before the start of the experiment some 5-10 fishes were placed in a rectangular aquarium with low depth of water (5 cm). Then 50-100 special food pellets containing any one of the test substances were introduced in the middle of the aquarium and the acts of bites the pellets were recorded for 5-10 minutes. This indicator was regarded as the extra-oral taste response of the fish since sturgeons grab only after preliminary touching of the pellet with barbels bearing external taste buds. After the experiment the remaining pellets were counted. As criterion reflecting the intensity of the intraoral taste response we used the percentage of consumed pellets per number of bites acts. The size of the aquarium, water level, the number of fish, the number of placed pellets and their size and shape, the duration of the experiment were the same in all experiments of the same series.

The pellets were prepared from either starch (12%) or agar-agar (2%) gels. After dissolving, starch or agar-agar solutions were added the day and one of test substances. Shortly before the experiment pellets were cutted out from cool gel using stainless steel tube. The pellet size was altered in accordance with fish body length. A detailed outline of the techniques employed is presented before (Kasumyan, 1992; Kasumyan and Kazhlaev, 1993; Kasumyan and Sidorov, 1995).

Free amino acids (l-isomers), water extracts and exometabolites of live daphnia, *Daphnia pulex,* or chironomid larvae, *Chironomidae,* two sturgeon's artificial feeds (ST-4Az, ST-07) and their 17 components (fish meal, krill meal, squid meal, PVF yeast, etc.) were used as olfactory stimuli. Free amino acids (l-isomers), classical taste substances and water extract of live chironomids larvae and were used as taste stimuli.

Results

Olfaction. The water extract and exometabolites of living food organisms were strong olfactory ecitants provoking the fish to display the specific food search response. This response is rather similar by its manifestation in the all five sturgeon species and consists in quick (after a few seconds) transition of the specimens to swimming only near the aquarium bottom, in the zone where the maximum concentrations of the food odor are created. Having sunk to the bottom the fish starts moving actively along searching trajectories in the form of circles and "S"-shape loops, their size being similar to the fish body length. In the process the fish touches surface of the bottom with their barbels and performs quick testing act - bites on the surface of

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the bottom. When moving along the searching trajectories , scouring was observed in the fish, i.e. successive removals by the body's fore part in a horizontal plane to both sides from the main direction of the motion. Scouring enabled the fish to cover a rather wide stripe, when moving, and owing to it, to investigate quickly and in detail bottom surface. After 30-60 seconds all specimens gathered near the compartment where the extract solution was added. The response was short by time (3-5 minutes) and strong depended on extract concentration. The chironomid larvae extract threshold was 10^{-4} g/l for juveniles of Russian sturgeon, Siberian sturgeon and stellate sturgeon.

This strong and remarkable behavioral response of sturgeons to extract of living food organisms is fully lost in olfactory deprivated specimens. So, the ability of sturgeons find the food by the chemical signals emanated from prey is based on olfaction.

Free amino acids are common chemical substances in exometabolites of various hydrobionts, solution many of amino acids induces food search behavior in different fish species. Only two amino acids from 20 were tested - glycine and alanine - exerted a behavioral effect for most sturgeon species were used: glycine (10^{-4} M) promoted an intense reaction in all five species of sturgeon, and alanine in the same concentration did so in the Russian sturgeon (8-10 cm in body length), Siberian sturgeon (8-12 cm) and stellate sturgeons (9-12 cm). For the hausen (15-20 cm) alanine was indifferent; in experiments with green sturgeon (30-40 cm) this amino acid was not used. The threshold concentrations of glycine and alanine were determined for Russian and stellate sturgeons at 10^{-6} M for both. The response pattern of sturgeons to effective amino acids was the same as to water extract of living food organisms. This is the clearly indicator for the signaling significance of these two amino acids. The anosmic sturgeons by cauterization of the olfactory rosettes completely loss the ability to react to glycine (10^{-4} M), showing that the response of the intact fish to the amino acid solutions is ensured exclusively by olfaction.

Taste. It was shown that a large proportion of free amino acids give a significant increase in the number of pellets grabs - 14 from 19 for the Siberian sturgeon (4-6 cm in body length), 16 from 21 for the Russian sturgeon (6-7 cm) and 19 (!) from 21 for the stellate sturgeon (6-8 cm). Common to all three sturgeon species were 12 amino acids although the correlation coefficient was high only for Russian-stellate sturgeon pair (r=0.84; p<0.001). The greatest effectiveness for extraoral taste sensitivity of these two fish species was displayed by aspartic and glutamic acids and cysteine and for Siberian sturgeon - asparagine, threonine and methionine. Only two amino acids - proline and tyrosine - were ineffective for all three species.

The spectra of the effective amino acids for the intraoral taste sensitivity were far narrower. Significant increase in the relative consumption of pellets was given only by 3 amino acids in stellate sturgeon, 6 - in the Russian sturgeon and 7 - in the Siberian sturgeon, 1 amino acid (alanine) having a deterrent effect for last species. Among amino acids stimulating consumption of the pellets there was nothing common to all three species nor were significant correlations discerned between species in term of the intraoral taste responses to the amino acids.

Comparison of the extraoral and intraoral taste responses within one species revealed a high correlation in the Russian (r=0.76; p<0.001) and lower correlation in the Siberian (r=0.47; p<0.05) and stellate (r=0.49; p<0.05) sturgeons.

Extracts of 13 from 17 components included into agar-agar pellets provoked the significant increase in the number of pellet bites for stellate sturgeon. Fish protein concentrate, PVF enzymated yeast and premixes had the highest activity for extraoral taste sensitivity of stellate sturgeon. Five components (PF-2V premix, krill meal, fish protein concentrate, Eprin yeast and

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couarsely cut soya-bean) had a stimulatory effect on intraoral taste receptors of fish. Fish meal stabilized by various antioxidant had different olfactory, and especially, extraoral and intraoral taste attractiveness.

Discussion

Thus it was establish that in different sturgeon species the olfactory spectra of the amino acids which evoking the search feeding behavior in fish largely concur both in breadth and composition. In sturgeons this spectrum is a very narrow, in most fish species now was investigated olfactory amino acids spectrum induced feeding behavioral responses is far broader (Jones, 1992). Glycine and alanine, effective in the case of the sturgeon, exert, as a rule, a behavioral effect also on a number of other species belonging to different systematic and ecological fish groups. However, for many fish species these two amino acids do not possess maximum activity as compared with other amino acids, for some species glycine and alanine are in behavioral terms inert.

Absence of appreciable species specificity of the behavior of fish to the odorous properties of free amino acids sharply contrasts with the fundamental species differences in the taste responses to free amino acids demonstrated by us in the same sturgeon species. The extraoral and intraoral taste sensitivity of the sturgeons sharply differ in the breadth of the spectrum of effective stimuli although they correlate well within the same fish species. The interspecies differences in the taste responses to the amino acids are considerable, especially in the case of intraoral taste sensitivity so suggesting clearly pronounced species specificity of the set of taste receptors and their tendency towards perception of different spectra of taste stimuli even in closely related species.

The result is that the same substances not only differ significantly in their taste qualities for the different sturgeon species but give responses diametrically opposed in character. For example, alanine displayed the most marked stimulating action in the stellate sturgeon, whereas it had a deterrent effect on the Siberian sturgeon including significant rejection of the captured pellets with this amino acid. Aspartic acid was one of the most palatable amino acid for Russian sturgeon and, in the same time, was ineffective stimuli for Siberian and stellate sturgeon.

The species specificity is the highest for the olfactory system followed by the external and intraoral taste systems. The biological significance of the different degree of definition of the species specificity in the olfactory and taste sensitivity of sturgeons to chemical stimuli resides in the different roles of these two types of chemosensory systems in the feeding behavior of fish. It is known that olfaction is a distant sense organ ensuring orientation and search by the fish for remote sources of the odor (Atema, 1980; Pavlov and Kasumyan, 1990). Many of free amino acids form part of exometabolites of hydrobionts and these substances are the natural odor markers of food organisms. The resemblance of the olfactory sensitivity to free amino acids in sturgeons like in many other fish species leads of different species to the site favorable for feeding. Then, in the next stages of the feeding behavior on the basis of taste reception the quality of the prey and its correspondence to the species food requirements of the fish are more finely evaluated. The high degree of species specificity of extraoral and, especially, intraoral taste spectra in sturgeons reflects the different food specialization of the fish species and the difference in the spectra of the objects of feeding preferred by them.

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THE EFFECT OF DENSITY ON THE MANIFESTATION OF

WHITE STURGEON IRIDOVIRUS DISEASE

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Previous investigations have reported the presence of the white sturgeon iridovirus (WSIV) in cultured white sturgeon Acipenser transmontanus from the lower Columbia River in Oregon, the Snake River in southern Idaho and the Kootenai River in northern Idaho (LaPatra et al. 1994). In Oregon, WSIV was consistently detected in young sturgeon that were progeny from Columbia River adults and cultured in river water but not detected in sturgeon cultured in well water. In Idaho, WSIV was detected in sturgeon that were progeny from wild Snake River and Kootenai River adults after being subjected to stressful conditions of low spring water flows and high fish densities (LaPatra et al. 1994). When densities were reduced and water flows increased, mortality subsided. These observations suggested that WSIV may occur in wild sturgeon and that the virus may be present in many Northwest populations due to the long life span of the species, migratory patterns, and continuity of the river systems. Additionally, since the disease appeared size(age)-specific and stress-mediated, fish culture management strategies could potentially be used to avoid or minimize epizootics.

A study was conducted at College of Southern Idaho (CSI) in Twin Falls to examine the effects of density on the manifestation of WSIV disease using the 1993 brood year Snake River white sturgeon (LaPatra et al. 1994). Replicate groups of sturgeon (mean weight 134 fish/lb or 3.4 g, 5 months old) were stocked at three densities in fiberglass aquaria with 11.1 ft^3 of volume and spring water flows of 8 gpm (Table 1).

Table 1. Total fish, cumulative weight, and loadings of sturgeon in duplicate groups cultured at three different densities.

| Density Designation	Low	Medium	High
Number of fish	300	600	1,000
Total weight (Lbs)[a]	2.2 (1020 g)	4.5 (2040 g)	7.5 (3400 g)
Lbs / ft^3	0.2 (91 g)	0.4 (182 g)	0.7 (318 g)
Sturgeon / ft^3	27	54	90

[a] Lbs = pounds.

Sturgeon mortality was monitored daily and specimens were fixed in neutral buffered formalin for histologic analysis (Humason 1979). Additional morbid animals were collected when mortality increased and submitted for viral isolation. Mortality ranged between 1-4% during the first 6 weeks and densities increased as sturgeon grew (Table 2).

Table 2. Fish size, mortality, loadings, and densities in sturgeon after being cultured for 42 d.

Density Designation	Low	Medium	High
Mean weight	10.3 g	9.4 g	8.6 g
Cumulative mortality	2.7%	1.3%	3.6%
Lbs / ft^3	0.5 (227 g)	1.1 (499 g)	1.6 (726 g)
Sturgeon / ft^3	22	53	84

ᵃ Lbs = pounds.

In the following 17 d period WSIV was diagnosed in all groups and mean cumulative mortality increased to 7%, 20%, and 57% in the low, medium, and high density groups, respectively.
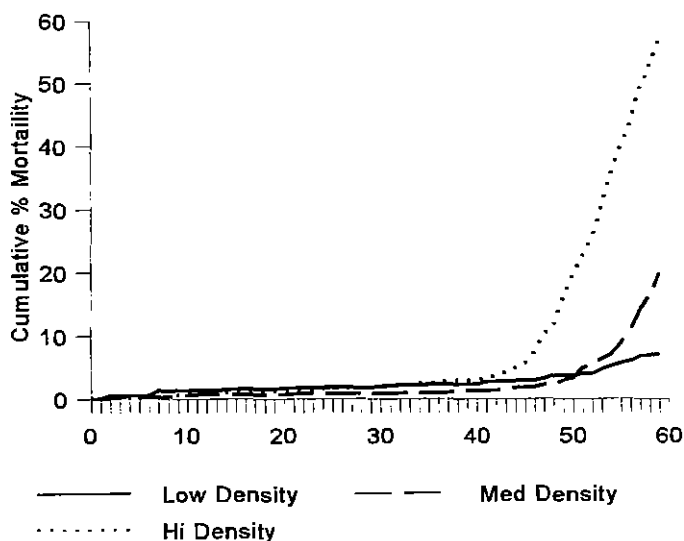
Cumulative percent mortality of the replicates was analyzed by analysis of variance on transformed (arcsin √percentage) data (Snedecor and Cochran 1967). No significant (p > 0.05) differences were observed among sturgeon held at different densities during the initial six week period. However, 17 d later cumulative mortality in the high density group was significantly (p > 0.05) different than mortality detected in the other treatments. The high and medium density groups were subsequently

divided into additional aquaria in an attempt to alleviate stressful conditions and minimize mortality. Test groups were monitored for an additional four weeks. Cumulative mortality in the medium and high density groups increased to 64% and 94%, respectively. Mortality also increased in the low density groups that had not been divided but was less (26%). Although mortality did not subside in the medium and high density groups after the disease appeared and densities were reduced, sturgeon maintained at the lowest density throughout the test period exhibited substantially less mortality to WSIV. These results suggest that maintaining low sturgeon densities in fish younger than one year may be a prudent strategy for minimizing mortality caused by WSIV.

In cooperation with Idaho Department of Fish and Game and the Kootenai Tribe of Idaho another density study using a similar experimental design and protocol was initiated at the Clear Springs Foods (CSF) Research Laboratory using the 1993 brood year Kootenai River white sturgeon (Apperson and Anders 1989). Groups of sturgeon (mean weight 126 fish/lb or 3.6 g, 5.5 months old) were stocked at high (0.7 lb/ft^3) or low (0.2 lb/ft^3) densities, previously tested at CSI. Fish were cultured in aquaria that received ultraviolet-light disinfected spring water. Triplicate high density groups and replicate low density groups were tested. Sturgeon exhibited no signs of WSIV or abnormal mortality immediately after transfer from the Kootenai Hatchery. However, after 36 d mortality increased to 10 - 18% (7/69 - 14/80) and 14 - 18% (39/281 - 49/272) in the low and high density groups, respectively, and WSIV was detected in morbid fish examined from each treatment. The presence of the virus was confirmed by electron microscopy. Sturgeon left at the Kootenai Hatchery (Bonners Ferry, Idaho) showed no evidence of WSIV. Although fish density did not appear to effect occurrence of WSIV or cumulative mortality, the results again suggested that a stressor (e.g. handling and transport) in subyearling sturgeon may enhance manifestation of WSIV disease.

Five months later the remaining fish at the Kootenai Hatchery were moved to the University of Idaho Aquaculture Research Institute. Approximately 1,840 sturgeon (mean weight 151 fish/lb or 3 g, 10.5 months old) with no detectable WSIV or abnormal mortality were transported at 8°C and acclimated to 15°C upon receipt. These fish were divided into six aquaria on a recirculation system supplied with chlorinated - dechlorinated well water and cultured at very low densities (0.05 - 0.07 lb/ft^3). During the first 10 d mortality among the six groups ranged from 1-7% and cumulative mortality was 2.7% (50/1829). However, over the next 60 d temperatures ranged from 12 - 19°C and cumulative mortality increased to 58% (1,064/1,838). Morbid animals exhibited signs of abdominal distention and emaciation and were diagnosed as WSIV-positive. In this case, densities again did not appear to be a factor in predisposing fish to a WSIV epizootic but handling, transport, and temperature may have been involved. Temperature stress may also have been a factor in manifestation of WSIV disease in siblings studied at CSF. These fish were transported approximately 14 h at 2-6°C, acclimated to 14°C over a 6 h period, and cultured at a constant temperature of 15°C.

As observed in previous studies, subyearling sturgeon appear to be predisposed to WSIV disease by stressors including densities, adverse environmental conditions (e.g. acute temperature fluctuations), and handling (LaPatra et al. 1994). Results from these studies further substantiate the potential for egg-associated and waterborne transmission of WSIV. Although the pathogen may be present, disease may not occur until certain stressors at critical life stages are confronted. We suspect that in each case described in this and previous reports the source of virus infection in cultured juvenile sturgeon was wild sturgeon either caught from the river and used for broodstock or present in the hatchery water supply (Hedrick et al. 1992; LaPatra et al. 1994). However, definitive evidence supporting this hypothesis has yet to be obtained.

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ANTIGENIC AND ULTRASTRUCTURAL CHARACTERISTICS OF EPITHELIOCYSTIS INFECTION IN CULTURED WHITE STURGEON

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Introduction

Epitheliocystis disease is a common condition of a putative infectious etiology that has been described in various teleosts (Paperna and Sabnai, 1980; Wolf, 1988; Turnbull, 1993; Fryer and Lannan, 1994). The descriptive term has been derived from the appearance of epithelial lesions manifested secondary to infection. Microscopically, progressive enlargement of infected epithelial cells subsequently results in the formation of spherical cysts that are circumscribed by an eosinophilic, hyaline capsule. Infected cells contain coccoid or coccobacillary organisms that complete a pleomorphic life cycle with specific morphological characteristics dependent on the stage of intracellular development. Development of the organism is similar to the chlamydiae (Ward, 1988) and proceeds from small, rigid, infectious forms to larger, pleomorphic, non-infectious forms that divide by fission to produce a new generation of infectious daughter cells. Taxonomic classification of the epitheliocystis agent has been speculative due to the inability to isolate the organism *in vitro*. Clinical disease has been attributed to respiratory distress secondary to gill epithelial hyperplasia and excessive mucous production although this association remains empirical. The present report expands the species catalogue of infection and presents antigenic and ultrastructural similarities between the white sturgeon (*Acipenser transmontanus*) epitheliocystis agent and the chlamydiae.

Materials and Methods

The white sturgeon examined were subyearlings (11 months, 250-300 g) from a population maintained at a private culture facility in southern Idaho. The population was divided into seven 1.0 m^3 aquaria with 50-75 fish per enclosure that were supplied with ambient, 13.5 C, non-recirculating, spring water at 45.4 liters per minute. Fish were fed a commercial soft-moist pellet at 2% body weight per day with a feed conversion of 1:1.7-1:1.9. Diagnostic efforts were initiated due to a 4-8% mortality (3-4 fish per enclosure) that was considered abnormal for this age-class under these culture conditions. Moribund fish were randomly selected from the affected population and fixed in 10% neutral-buffered formalin. Tissue sections were cut to 5 µm and stained with hematoxylin and eosin (HE), periodic acid-Schiff (PAS), Brown and Brenn (BB), Macchiavello and Gimenez reagents. Selected areas of gill tissue were procured from the paraffin blocks for transmission electron microscopic examination. A standard peroxidase-antiperoxidase (PAP) immunohistochemical staining procedure using a primary monoclonal antibody specific for the chlamydial genus-specific lipopolysaccharide (LPS) antigen was used to demonstrate the expression of chlamydial antigen in infected epithelial cells of the gills.

Results

Cellular lesions typical of epitheliocystis infection were observed in the gills. Infected branchial epithelial cells contained the coccoid to coccobacillary epitheliocystis organisms that appeared as

cytoplasmic inclusions composed of a fine, homogeneous, dense, basophilic, granular material (Figure 1). The infected cells were variably enlarged with spherical to oval profiles and randomly distributed throughout the branchial epithelium. The inclusions were circumscribed by a variable amount of host cell cytoplasm that consequently became less abundant with progressive enlargement of the cytoplasmic inclusions. The nuclei of infected cells were initially enlarged and eccentrically located with a single prominent nucleolus or multiple nucleoli but became attenuated and progressively less prominent subsequent to the progressive enlargement of the cytoplasmic inclusions. The cytoplasmic inclusions stained positive with Macchiavello but negative with Brown and Brenn, PAS and Gimenez stains. Expression of chlamydial antigen was demonstrated within the cytoplasmic inclusions using a standard peroxidase-antiperoxidase immunohistochemical technique and a primary murine monoclonal antibody specific for the chlamydial genus-specific antigen (Figure 1). Host reaction was absent or limited to a mild epithelial hyperplasia of the gills although the latter could not be definitively attributed to the infection.

Three stages of coordinated intracellular development were recognized by electron microscopy. Regardless of the developmental stage, the organisms were delimited by a cell membrane that was composed of two electron-dense zones separated by an electron-lucent layer. A variable number of discrete cytoplasmic condensations that were not membrane-bound and of variable density dependent on the stage of development were also a common feature. The reticulate bodies were oval to spherical and 0.4-0.8×0.5-1.4 µm but often exhibited a pleomorphic and convoluted appearance due to variable membrane invaginations and evaginations suggestive of uneven fission and budding. A few enlarged reticulate bodies with a maximum size of 2.2×3.7 µm had an exaggerated pleomorphic and convoluted appearance and contained multiple cytoplasmic condensations. The pleomorphic appearance of the enlarged reticulate bodies was due to multiple membrane evaginations consistent with the process of budding. Separate host cells contained intermediate bodies that were spherical to oval and 0.2-0.4×0.3-0.6 µm although often observed in the process of apparent uneven division. These cells contained a single, compact, cytoplasmic condensation that were completely circumscribed by an electron-lucent zone or halo. The presence of a cap or plaque composed of hexagonally arrayed, fibrillar, surface projections was initially recognized at this stage. Tangential sections demonstrated that the cap was composed of up to 30 surface projections. An homogeneous population of elementary bodies that were oval and 0.3-0.4 µm also occurred separately in individual host cells (Figure 2). This developmental stage had a single, dense, compact, eccentrically located, cytoplasmic condensation and a single cytoplasmic vacuole that was not membrane-bound. The hexagonally arrayed fibrils were more distinct at this stage and occurred opposite to the eccentrically located, cytoplasmic condensation. The cell membrane associated with the fibrils constituted approximately 25% of the cell circumference and displayed a prominent electron-density relative to the remainder of the cell membrane.

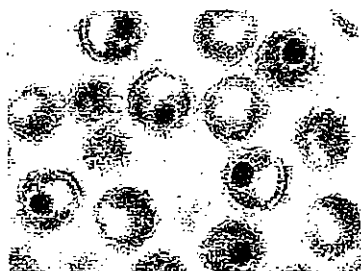Figure 1. Infected branchial epithelial cells positive for chlamydial LPS antigen.

Figure 2. Epitheliocystis elementary bodies with characteristic nucleoid and hexagonally arrayed fibrils.

Epitheliocystis disease is a common condition that has been observed in various teleosts as an incidental finding (Paperna and Sabnai, 1980; Wolf, 1988; Turnbull, 1993). This incidental form of the disease has been characterized as a non-lethal or chronic condition due to the apparent mild infection and associated host response that is typically absent or limited to a mild epithelial hyperplasia. A severe form of the disease characterized as an hyperinfection may result in a diffuse, severe, proliferative, epithelial hyperplasia often with branchial lamellar fusion and excessive mucous production (Paperna, 1977; Rourke et al., 1984; Bradley et al., 1988).

Previous attempts to classify the epitheliocystis agent have been based on *in situ* ultrastructural characteristics of the organism due to the inability to culture and isolate the organism *in vitro*. Development of the organism is similar to the chlamydiae that proceed from small, rigid, infectious forms to larger, pleomorphic, non-infectious forms which divide by fission to produce a new generation of infectious daughter cells (Ward, 1988). The morphological characteristics observed in the present report were consistent with the features that occur during these various stages of chlamydial development.

Five morphological stages of epitheliocystis development have been described in various species and referred to as the initial or reticulate bodies, elongated cells, round cells, small cells and head and tail cells (Turnbull, 1993). The initial or reticulate body designation has been used to describe the 0.7 to 1.25 μm, irregularly shaped, epitheliocystis cells due to the morphological similarities of these cells with the 0.5-1.3 μm reticulate bodies characteristic of early chlamydial development (Rourke et al., 1984; Bradley et al., 1988; Desser et al., 1988). The reticulate bodies observed in this report were similar to these previous descriptions. Furthermore, the pleomorphic and convoluted appearance that was often highly exaggerated and suggestive of division has been previously been described for epitheliocystis in other host species (Desser et al., 1988) and for the chlamydiae (Ward, 1988). Two forms of elongated cells have been described for the epitheliocystis agent (Paperna et al., 1981). The primary long cells (PLC) have a reported maximum size of 0.3-0.6 x 7.5 μm and contain an electron-lucent matrix with peripheral ribosomes and loose, fibrillar, cytoplasmic condensations. The intermediate long cells (ILC) are produced from the PLC by binary fission and budding but are usually smaller with a reported range of 0.3-0.6 x 1.0-2.0 μm and contain more dense cytoplasmic condensations relative to the PLC. These elongated cells may represent morphological variants of the reticulate body in these host species although their structure and mode of division has been reported to resemble the rickettsial giant or initial body (Paperna et al., 1981).

Epitheliocystis round cells have been observed in various host species and referred to as intermediate bodies due to the shared ultrastructural similarities with the intermediate developmental stage of the chlamydiae that occurs in transition between the reticulate body and elementary body forms (Paperna and Sabnai, 1980; Wolf, 1988; Turnbull, 1993). These round cells have a reported size range of 0.3-1 μm and are similar to the 0.2-0.4 x 0.3-0.6 μm spherical to oval, intermediate bodies observed in this study. The final and infective stage of epitheliocystis development has been assumed to be the designated small cell due to the similarity with the chlamydial elementary bodies (Turnbull, 1993). These small cells are less than one micrometer in diameter and contain an electron-lucent vacuole, a dense cytoplasm and a dense, compact, cytoplasmic condensation that is surrounded by an electron-lucent zone or halo similar to the 0.3-0.4 μm oval elementary bodies observed in this report. This dense, compact, cytoplasmic condensation or nucleoid has been considered a hallmark of the chlamydial elementary body (Costerton et al., 1976). The cap of hexagonally arrayed, fibrillar, surface projections that occurred opposite to the location of the dense, compact, matrix condensation as described in this report has also been observed in the later stages of epitheliocystis development in the brown bullhead (*Ictalurus nebulosus*; Desser et al., 1988). These surface projections are similar to the unique structures described for the chlamydial elementary bodies that are considered requisite for inclusion in this group (Gregory et al., 1979). Although the function of these surface projections remains speculative, their hollow and cylindrical structure may be indicative of a transmembrane pore that connects the interior of the cell to the external environment or that is involved in interchlamydial communication (Louis et al., 1980; Matsumoto et al., 1982). The presence of these surface projections in the later stages of development also suggests a role in the attachment to host epithelial cells prior to infection.

Head and tail cells have been previously observed in only two species of salmonids (Rourke et al., 1984; Bradley et al., 1988). These cells reportedly exhibit the common epitheliocystis features and

are comprised of an elliptical, 0.3-0.4 μm head region that contains a dense cytoplasmic condensation and a tail region up to 0.3 μm in length with a terminal 0.125 μm expansion. These cells reportedly resemble a degenerative form of the rickettsial organism, *Coxiella burnetti* (Weiss and Moulder, 1984), and may represent failed or delayed division, an artifact or a form unique to these host species (Turnbull, 1993). However, cells with this characteristic morphology were not observed in this study.

Attempts to classify the epitheliocystis organism based on ultrastructural morphology have been unsuccessful due to the limitations inherent in these methods that include sampling error and the inability to accurately assess the variability in epitheliocystis development that may occur with strain differences or with differences in the host species influence. However, inclusion in the Chlamydiales has been suggested since the various developmental stages share ultrastructural similarities with members of this group (Paperna et al., 1981; Desser et al., 1988). Placement in the Rickettsiales has also been proposed based on the similarities in morphology and mode of division of the epitheliocystis PLC with the rickettsial initial or giant body (Paperna et al., 1981). This argument has been supported by the failure to detect epitheliocystis expression of the chlamydial genus-specific LPS antigen in previous investigations (Bradley et al., 1988; Turnbull, 1993). However, positive demonstration of chlamydial antigen in the present study provides further evidence that the epitheliocystis organism is related to members of the Chlamydiales.

The source of infection in the present report was not determined although transmission via fomites (Paperna, 1977) or through the water supply was considered the most likely explanation. The high-density culture of these sturgeon would further promote the transmission and exacerbation of infection within the population. The sturgeon examined in this study were randomly selected and may not have been representative of the severity of infection responsible for the low-level mortality in this population. Therefore, epitheliocystis infection cannot be ruled-out as the primary cause of mortality in this population especially since other infectious disease agents were not identified nor were water quality or other environmental parameters considered a limiting factor.

In conclusion, the ultrastructural characteristics of the epitheliocystis organism in these white sturgeon was similar to previous descriptions in other host species and expands the species catalogue of epitheliocystis infection. Ultrastructural characteristics similar to the chlamydiae in association with the immunohistochemical demonstration of chlamydial antigen within the cytoplasmic inclusions provides further evidence that the epitheliocystis agent is related to members of the Chlamydiales. Although the infection was considered mild to moderate and could not be definitively attributed to the mortality in this population, the institution of surveillance methods to detect and subsequently prevent the transmission of epitheliocystis infection in cultured populations should be considered. Likewise, the practicality and viability of alternative husbandry methods that reduce or eliminate the potential adverse impact of epitheliocystis infection especially in high-density operations need to be evaluated.

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Physiology and Pollution

THE EFFECTS OF HYPERCAPNIA ON BLOOD-GAS, ACID-BASE STATUS IN

THE WHITE STURGEON, *ACIPENSER TRANSMONTANUS*

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Abstract

Intensive culture of white sturgeon in facilities that incorporate oxygen injection and water reuse may be negatively affected by high dissolved water $[CO_2]$. Blood-gas, pH, plasma [catecholamine] and [cortisol] were measured in sturgeon subjected to hypercapnia (water PCO_2 = 40-60 mg/L and pH 6.5-7.0). Blood was sampled remotely (non-occlusive cannulation of dorsal aorta) at intervals to establish the time course of the physiological responses to hypercapnia. Chronic (72-96 h) environmental hypercapnia resulted in an uncompensated respiratory acidosis, hyperventilation, and elevated plasma catecholamine and cortisol titres.

Introduction

Current high-density culture of white sturgeon (*Acipenser transmontanus*), using O_2 injection and water reuse, results in high metabolically produced CO_2 concentrations (hypercapnia). The effect of environmental hypercapnia on arterial pH in some fishes is well documented: there is an initial increase in plasma $[CO_2]$ with a concomitant reduction in pH_a (Cameron and Randall 1972, Claiborne and Heisler 1986, Cameron and Iwama 1987). Teleostean fish exposed to environmental hypercapnia generally exhibit a graded respiratory acidosis which is partially compensated for by a significant elevation of plasma bicarbonate. The time course and magnitude of the compensation are variable and largely dependent upon non-bicarbonate buffering capacity and acid-base relevant transfer mechanisms (Eddy et al. 1977).

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We have shown that hypercapnia affects the functioning of the chondrostean white sturgeon. Chronic, sublethal, hypercapnic exposure significantly reduced growth of young-of-the year white sturgeon, via reductions in foraging behavior, presumably mediated by severe arterial acid-base, blood-gas disturbances (Crocker and Cech, submitted). Furthermore, we have demonstrated that environmental hypercapnia [water $PCO_2 = 40\text{-}60$ mg/L] significantly increases oxygen consumption rate and respiratory frequency in juvenile white sturgeon (Crocker and Cech, unpublished data). The present work was conducted to address the following questions: (1) Does exposure to hypercapnia cause acid-base disturbances in white sturgeon? and (2) What is the endocrine response to acute hypercapnic exposure? The primary objective of this study was to monitor and describe the time course for the development and recovery of blood-gas, acid-base disturbances associated with acute environmental hypercapnia. A second objective was to characterize the "stress" response in white sturgeon acutely exposed to sublethal environmental hypercapnia.

Materials and Methods

Animals
Sibling juvenile white sturgeon (0.8-1.4 Kg) were transferred from the UC Davis Aquaculture and Fisheries Program Facility (located at the Institute of Ecology) to the Wildlife, Fish, and Conservation Biology Department's fish ecophysiology laboratory and placed in 1.3 m diameter fiberglass tanks (750 L) receiving a continuous flow of aerated, dechlorinated well water. They were fed twice daily (Silvercup Trout Pellets), were kept on a natural photoperiod, and allowed at least two weeks to recover from any transport-related stress.

Surgical preparation
After the acclimation period, a randomly selected fish was dip netted and placed into a buffered (pH 7.0 using N_aHCO_3) anesthetic water bath (MS-222; 6g/30L) until ventilatory movements ceased. It was placed onto an operating table (in dorsal recumbency) and artificially ventilated with an anesthetic solution (MS-222; 2g/30L). A 17 ga., 75 mm hypodermic needle was inserted into the hemal arch (posterior to the cloaca) and the dorsal aorta was non-occlusively cannulated using a 1-m length of PE-50 tubing filled with heparinized saline (100 IU/ml). The cannula was sutured at its point of entry and then anchored onto the lateral aspect of the body wall (Ethicon 3.0 silk). Recovery was rapid (< 5 minutes) and initiated by artificial ventilation using aerated, anesthetic-free water. Once ventilatory activity returned, the fish was placed into a cylindrical (30 L) plexiglass, flow-through chamber, receiving a continuous flow of aerated, unchlorinated well water, and allowed at least 24 hours to recover from surgery. Each chamber was partially covered with black plastic to shield the animal from laboratory activity. Cannulae were flushed with heparinized saline (100 IU/ml) every day to minimize clotting at the tip and to avoid the formation of thrombi. Post-experimental examination of the cannulation position was used to reveal whether or not significant tissue damage resulted from the surgery.

Experimental design
Fish were randomly assigned to either the hypercapnic treatment (test) group or the normocapnic (sham) group. Test fish received water equilibrated with CO_2 gas which was delivered, using a gas mixing flowmeter (Cameron Instrument Co.), to a PVC stripping column (Fry 1951) mounted above the experimental area. For both treatment groups, blood samples (0.7 ml) were remotely withdrawn, via the cannula, at 0, 1, 2, 24, 48, 72, and/or 96 h after reaching the desired level of hypercapnia (test fish). After the 96 h sample, hypercapnia was terminated and the fish were returned to normocapnic water conditions. The sham fish endured the surgery and the blood sampling protocols but were not exposed to hypercapnia. Final blood samples (120 h) were taken for both treatment groups 24 h after terminating hypercapnia.

Water analysis

Water PO_2 and PCO_2 were measured with thermostatted electrodes (Radiometer PHM 3/E5046/D616 and E5036/D616, respectively). Water pH was measured with a hand-held pH meter (Corning PS-15). Water temperature was monitored daily using a mercury thermometer and maintained at 19.5 °C using chillers and submersible heaters and $[Cl_2]$ and $[NH_3/NH_4^+]$ were measured daily using a Hach (Cl_2 test kit) and a Chemetrics ammonia test kit (Ammonia 1 DCR), respectively.

Blood Analysis

Blood pH was measured with a thermostatted electrode (Radiometer PHM73/G297/K497) calibrated with temperature-corrected precision buffers (Radiometer). Blood PO_2 and PCO_2 were measured using the Radiometer thermostatted electrodes calibrated with humidified N_2 and air (PO_2) and with a 10% CO_2/ 90%air mixture (PCO_2). Blood hematocrit (percentage, after centrifugation after 3 min at 11,000 X g) and whole blood [lactate] (mM/L; YSI 2700 Select) were immediately measured. Blood [hemoglobin] was determined on 20-μl blood samples using a spectrophotometric assay kit (Sigma Kit 525). The remaining blood was centrifuged at 4,500 X g for 5 min and the plasma stored at -5°C for later analysis. Plasma $[HCO_3^-]$ was calculated using pH and PCO_2 data in the Henderson-Hasselbalch equation (Davenport, 1974) with constants published by Reeves (1976). Respiratory frequencies were determined visually by counting opercular movements.

Chemical Analyses

Plasma catecholamines (epinephrine and norepinephrine, nM/L) were analyzed at California State Polytechnic University, Pomona on a BAS (Bioanalytical Systems, Inc.) high performance liquid chromatography (HPLC) system with electrochemical detection (see Stiffler et. al., 1990). The plasma samples were preserved with the antioxidant sodium metabisulfite (1 mM, 50% of volume), stored at -70°C and transported to Pomona on dry ice. Catecholamines were extracted on aluminum oxide at pH 8.6 (1.5 M hydroxymethyl-aminomethane (Tris)-EDTA buffer), the aluminum oxide was washed twice with ice-cold glass-distilled water, and the catecholamines were then eluted with 0.1 M $HCLO_4$. The internal standard, 3,4-dihydroxybenzylamine was placed in all standards and samples to control for minor variations in extraction-elution efficiency between tubes.

The samples were then centrifuged and the eluate injected into the HPLC system circulating a mobile phase consisting of 0.15 M monochloroacetic acid, 1 mM Na_2EDTA, and 1 mM sodium octylsulfate, in 1.5% acetonitrile, pH 3.0. The temperature in the system was maintained at 35 °C. The column was a 7-μm BAS Phase II ODS reverse-phase column, and the flow rate was 1.0 ml/min. Plasma cortisol (ng/ml) was determined at the CPSU, Pomona, laboratory, using a commercial assay kit (Coat-A-Count kit; Diagnostic Products Corp.), and a gamma counter (Beckman Instruments).

Statistical Analyses

Data presented are means$\pm$ SEM. Analysis of variance (ANOVA) was used to compare means between treatment groups. When assumptions for parametric tests were violated, the Kruskal-Wallis one way ANOVA on ranks was used. A multiple comparison procedure (Dunnett's Method) was used to compare means between groups, and pairwise multiple comparisons within groups were made using Dunn's Method. $P<0.05$ was used as the level of significance.

Results

Environmental hypercapnia (water PCO_2 : 40-60 mg/L, pH: 6.5-7.0) resulted in arterial hypercapnia (Fig. 1) and an uncompensated respiratory acidosis (blood pH significantly reduced
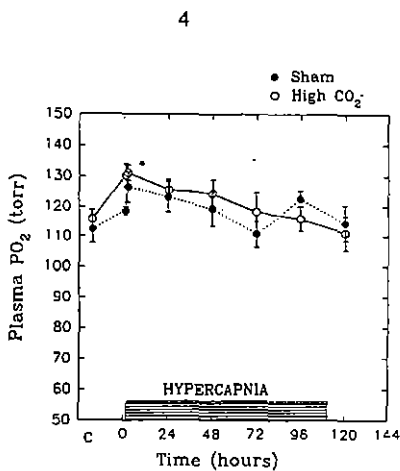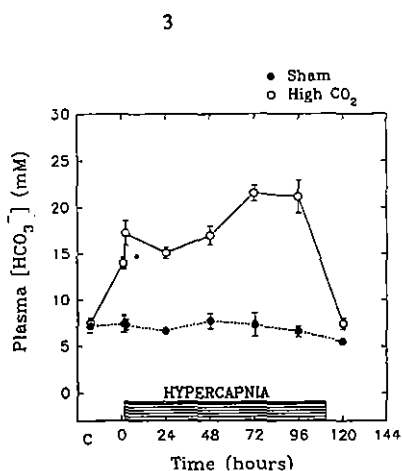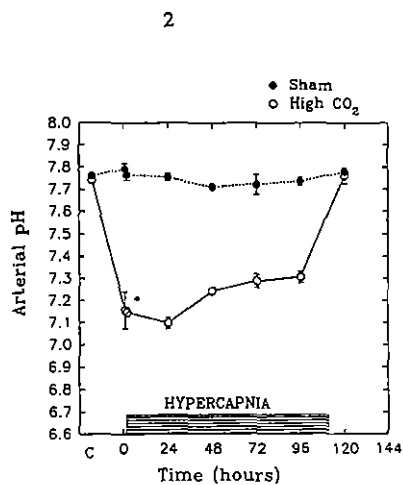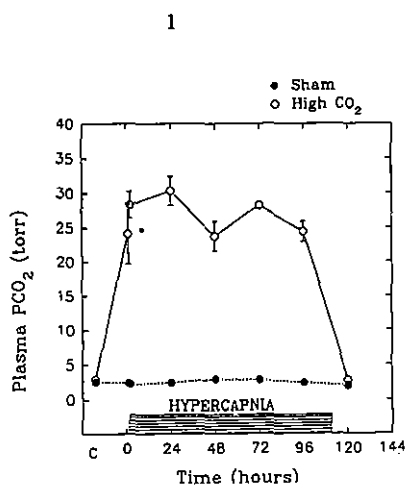
Figures 1-4. Effect of hypercapnia on arterial PCO_2 (Fig. 1), pH (Fig. 2), $[HCO_3^-]$ (Fig. 3), and PO_2 (Fig. 4). * Indicates a significant difference in test fish between hypercapnic and pre-hypercapnic (normocapnic control) sturgeon (C= control).

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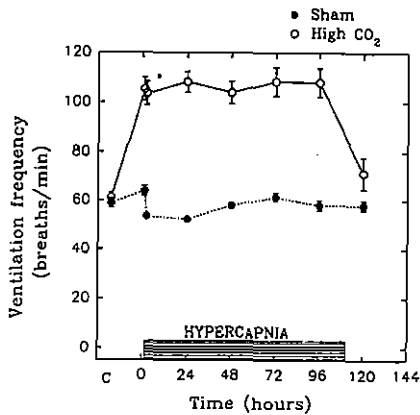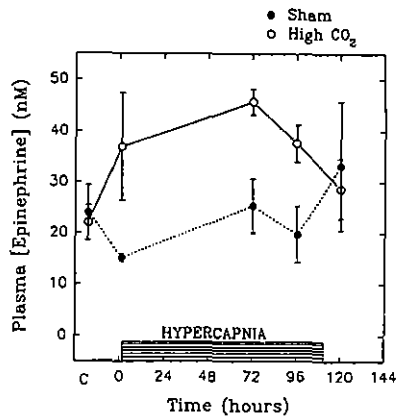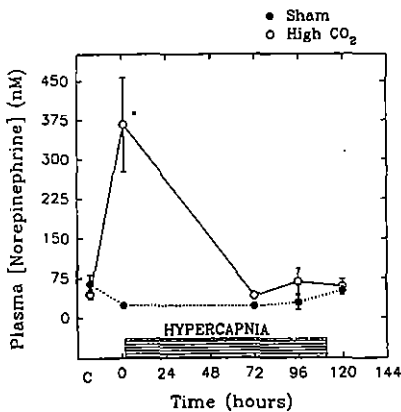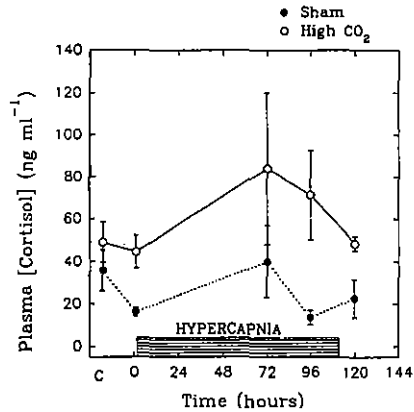
Figures 1-4. Effect of hypercapnia on ventilatory frequency (Fig. 5), plasma epinephrine (Fig. 6), plasma norepinephrine (Fig. 7), and plasma cortisol (Fig. 8). * Indicates a significant difference in test fish between hypercapnic and pre-hypercapnic (normocapnic control) sturgeon (C= control).

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by ca. 0.5 units) that persisted throughout the 96 h exposure period (Fig. 2). [HCO$_3^-$] increased during hypercapnia (Fig. 3) while PO$_2$ increased initially with hypercapnia but then returned to near control levels after 24 h (Fig. 4). Ventilatory frequency increased significantly during the entire hypercapnic period (Fig. 5). Plasma [epinephrine] and [cortisol] were not significantly elevated in the test fish (compared to the sham fish), whereas plasma [norepinephrine] showed a transient spike immediately after the onset of hypercapnia (Figs. 6, 7, 8). After returning the fish to normocapnic conditions (96- 120 h) there was a significant increase in blood pH and a decrease in PCO$_2$ ventilatory frequency, and plasma [bicarbonate]. Water [Cl$_2$] and [NH$_3$/NH$_4^+$] were always below 0.01 ppm and 0.2 ppm, respectively.

Discussion

In juvenile white sturgeon, environmental hypercapnia results in a uncompensated respiratory acidosis with a concomitant significant increase in ventilatory frequency. Teleostean and elasmobranch fishes typically increase plasma bicarbonate during acidosis to regulate blood pH. In the present study, arterial PCO$_2$ and plasma [HCO$_3^-$] were significantly elevated above pre-hypercapnic levels during hypercapnia (Figs. 1 and 3) , but blood pH did not return to pre-hypercapnic levels while the hypercapnia was maintained (Fig. 2). The water PCO$_2$ level (ca. 20 - 25 torr), the decrease in pH$_a$ at the onset of hypercapnia, and the lack of compensation relative to the reduction of pH$_a$, was virtually identical to that of severely hypercapnic carp (*Cyprinus carpio*)(Claiborne and Heisler 1986). In contrast, the marine teleost *Conger*, almost completely compensates for the reduced pHa by accumulating bicarbonate when exposed to mild (ca. 8 torr) hypercapnia (Toews et al. 1983). Similarly, Eddy et al. (1977) demonstrated considerably rapid and effective metabolic compensation in rainbow trout (*Oncorhynchus mykiss*) suffering a respiratory acidosis (PCO$_2$: ca. 15 torr). Claiborne and Heisler (1986) showed that the carp achieves complete pH compensation during mild hypercapnic exposure (ca. 8 torr) but severe hypercapnia (ca. 35 torr) causes disruptions of acid-base regulatory mechanisms beyond their compensatory ability. Similarly, Eddy et al. (1979) demonstrated that rainbow trout, subjected chronically to water of high CO$_2$ content (35-40 torr PCO$_2$) suffered from an uncompensated respiratory acidosis. While white sturgeon cannot metabolically compensate for severe hypercapnia (this study), Cameron and Iwama (1987) demonstrated that channel catfish, (*Ictalurus punctatus*) were able to achieve metabolic compensation (>70%) via bicarbonate accumulation when exposed to severe environmental hypercapnia (30-58 torr PCO$_2$).

While hyperventilation is only effective at reducing P$_a$CO$_2$ when the inspired-arterial PCO$_2$ difference is large (Heisler 1993), the immediate, persistent, elevated ventilatory frequency reported here (Fig. 5) may be associated with a significant Bohr shift in white sturgeon blood associated with the increase in P$_a$CO$_2$ (Crocker and Cech, unpubl. data). Since the metabolic compensatory processes that regulate pH$_a$ in fish during hypercapnia do not typically involve hyperventilation (Cameron and Randall 1972), the increase in gill ventilatory frequency may increase arterial O$_2$ (Fig. 4). The hyperventilation reported for the white sturgeon is in contrast to the response of the larger spotted dogfish (*Squalus stellaris*) exposed to mild hypercapnia (ca. 5 torr; Randall et al. 1976) but consistent with that of rainbow trout (*Oncorhynchus mykiss*) (ca. 15 torr; Eddy et al. 1977, Thomas and Le Ruz 1982, Thomas et. al. 1983). The PaO$_2$ did not remain elevated throughout the entire period of hypercapnia in our study. While the magnitude of the acid-base disturbance, during severe hypercapnia, is evident, the temporal aspects of compensation of the acidosis for white sturgeon remains elusive. The lack of apparent adverse effects after 96 hours of hypercapnic exposure (no mortalities) presumably demonstrates the white sturgeon's resilience to this environmental perturbation.

Circulating catecholamines may be associated with maintaining arterial PO$_2$ in white sturgeon during chronic environmental hypercapnia. Catecholamines interact with β receptors in the

heart (activating positive inotropic and chronotropic cardiac states), gills (to mediate branchial vasodilation), and α receptors in the peripheral vasculature (presumably to increase oxygen delivery to certain tissues) (Moyle and Cech 1996). In the present study, the circulating plasma [norepinephrine] was always higher than plasma [epinephrine] (Figs. 6 and 7). According to Randall and Taylor (1989), increased plasma [catecholamine] will accompany the increase in ventilation seen with hypercapnia. This may offset the effects of decreased blood oxygen affinity at the sturgeon's gills (Bohr shifts) and may account for the initial increase in arterial PO_2 at the onset of hypercapnia. The resting plasma catecholamine levels reported in this study are higher than those reported for the Siberian sturgeon (*Acipenser baeri*) exposed to hypoxia (Maxime et al. 1995) but similar to resting catecholamine levels reported for spotted dogfish (25.6 nM/L and 32.1 nM/L; epinephrine and norepinephrine, respectively) by Butler et al. (1978), and to resting levels of 28.8 nM/L and 13.2 nM/L in the Atlantic cod, (epinephrine and norepinephrine respectively; Wahlqvist and Nilsson 1980). During stress, cortisol mobilizes energy reserves (Schreck 1981) and activates RBC adrenoreceptors (Reid and Perry 1991). This response is typically latent with respect to the catecholamine response. Mean resting plasma [cortisol] in the present study was higher than those reported by Maxime et. al. (1995) for *Acipenser baeri* (Fig. 8).

In summary, white sturgeon show essentially no compensation to hypercapnia-induced respiratory acidosis, compared with elasmobranchs and teleosts previously investigated. Further research is needed to determine if this pattern is characteristic of chondrosteans in general.

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DEVELOPMENTS OF THE CNS OF STURGEON FISHES

GROWN UNDER DIFFERENT ECOLOGICAL CONDITIONS

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Conditions of the sturgeon youth fishes, growing at fish farms differ significantly from the natural ones, this influences greatly the huching viability. The factor determining adaptation properties of the youth is the Central Nervous System (CNS) development. It has been shown , that the sturgeon youth fishes grown in reservoirs and ponds (which hydrologic conditions like the natural ones) differ greatly from each other in respect to bechavioral and reflectory reactions (Kasimov, 1980; Kasimov et al., 1986).

The present work gives for the first time a thorough neuro-morpho-physiologic analysis of the brain development in star sturgeon (*Acipenser stellatus Pallas, 1771*) youth fishes 30 to 52 days after hatching (A.H.) growing on the Astrachan fish farm. The hutchlings fishes were grown in standart concrete Reservoirs (3 m in diametre, 12 to 15 sm deep, permanent water flow, abundant forage) and in Ponds. (2 to 2,5 hectares, 2 to 2,5 m deep, pronounced water flow, scanty forage, predators) The hydrologic conditions of the ponds were similar to those in the star sturgeon distribution area in the Volga River.

The main attention was paid to analysis of the changes in the Telencephalon structure as far as namely in this CNS region the centres of all principal analyzer systems are situated, and the region itself influences at the fish behaviour (Northcutt,Braford 1980, Nikonorov, 1982, Obukhov,1993).

By application of automatic imagination analysing complexes IBAS (Germany) and MAGYSKAN-2AR (United Kingdom) and original PC programms the following features of the brain structure were studied: square of telencephalon hemispheres and their zones; neurone sizes and density of the cell distribution over the zones.
R. Nieuwenhuys' classification was used for the hemispher structure description. (Nieuwenhuys & Meek, 1990)

In Actinopterygian fishes telencephalon develops in a way fundamentally differs from that found in all other vertebrates. In the early stages of the brain development lateral

walls telencephalon evaginate, that leading to the formation of hollow (everted) cerebral hemispheres (Nieuwenhuys, Meek, 1990; Andreeva, Obukhov, 1991). The sturgeon telencephalon is in accordance to the general pattern typical for all Actinopterygian fishes. It consist of twin everted hemispheres, subdivided histologically into two regions: dorsal area **(D)** - pallium, and ventral area **(V)** - subpallium, situated in the dorso-lateral and ventro-medial position respectively (Figure 1).

Figure 1. Cytoachitecture of the adult sturgeon telencephalon.
    Nissl-stain transverse section through middle levels hemispheres. Abbreviations:
        D- area dorsalis telencephali (pallium); D.m., D.d-l., D.c. -medial, dorsolateral,central zones dorsal area; V - area ventralis telencephali (subpallium); V.d., V.v., V.l. - dorsal,ventral, lateral zones vental area; es - ependymal surface;   ms - meningeal surface;VM - ventriculus medialis; t.ch.- tela chorioidea;   z.lim.- zona limitas

In the pallium it is possible to note two longitudinal zones, which extend along the ependymal surface: dorsomedial (**D.m.**) and dorsolateral (**D.d-l**). These zones surround another more interior zone - dorsocentral (**D.c**). The greater part of the D.d-l. zones contains large neurons, wich are arranged a number of layers (8-12) parallel to the ependymal surface hemispheres. In the dorsomedial zone (D.m) the neurons are less regularly arranged, that those in the other zone' s pallium. Characteristic feature of central zone (D.c) are large size of neurons and lacking of the lamellated pattern.

In the subpallium medial surface is composed of the epenymus of the medial telencephalic ventricle (VM), its lateral surface is lined by the meningeal tissue. Dorsally it is joined to the area dorsalis (the separation between these two areas in marker by cell-free strip - zona limitans (z.lim). The general pattern organization of the ventral area in sturgeon species is very similar, is the results of the lack of an inversion in this regions. In subpallium three nuclei present: dorsal zone (**V.d**) - group larger neurons, part of its migrated a way from ependymal surface of hemispheres: ventral zone (**V.v.**)- groups of smaller cells, sittuated along ventricular surface and lateral zone (**V.l.**) - sparsely distributed cell groups in the regions, bordering the subminengeal region of telencephalon.

The specific functional role of earch of these areas and zones unknown, however physiological experiments have shown that in Actinopterygian specific telencephalic areas bind with the specific types of their behaviour At present time attempts to compare parts of the everted telencephalon Actinopterygian fishes with the same areas of the telencephalon of other vertebrates groups have been made, however this problem do not solve now. (Northcutt,Braford, 1980; Nieuwenhuys, Meek, 1990; Andreeva, Obukhov, 1991).

Tiny fishes (30 - 32 days old)
Tiny fishes of this age, grown in reservoirs possess extremely poor-developed telencephalon. The hemisphere square makes up 400 $\pm$12 of square units. The main bulk of neurones is recorded by the hemisphere surface, differentiation into zones is practically absent. (Figure 2.A). Small neurones predominate (Table 1.).

Table 1. Telencephalic neurone sizes (mcm ^{2}) in star sturgeons Acipenser stellatus grown under different conditions.

Age	Growth conditions	Pallium zones		
(days)		D.m.	D.d-l.	D.c.
30-32	reservoir	22.6 $\pm$ 1.4	23.9 $\pm$ 0.9	53.1 $\pm$ 2.1
	pond	48.4 $\pm$ 2.1	41.7 $\pm$ 1.8	77.2 $\pm$ 2.4
50-52	reservoir	28.9 $\pm$ 1.8	38.5 $\pm$ 2.1	78.9 $\pm$ 3.7
	pond	57.0 $\pm$ 2.1	53.7 $\pm$ 1.8	92.9 $\pm$ 3.0

In the tiny fishes of the same age, grown in pond, the similar hemisphere size (390 $\pm$111 square units) is accompanied with the apparent migration of neurones into telencephalon

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wall. Neurones sizes in the respective zones increase significantly (Table 1)The
zonal differentiation of hemisphere is more distinct (Figure 2C, 3C)
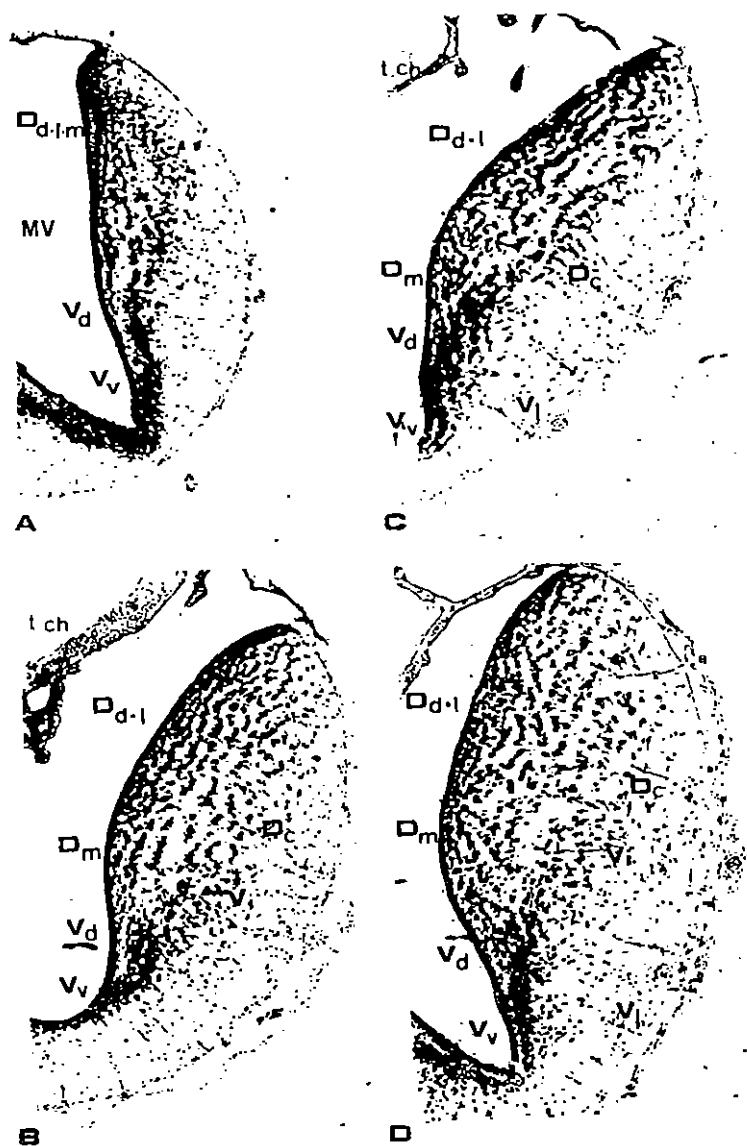
Figure 2. Cytoachitecture of the telencephalon of tiny star sturgeon (Acipenser stellatus P
grown in reservoirs (A,B) and ponds (C,D). Nissl-stain transverse sections.
A,C - 30-32 days A.H.; B,D - 50-52 days A.H., abbreviations as on Fig.1.
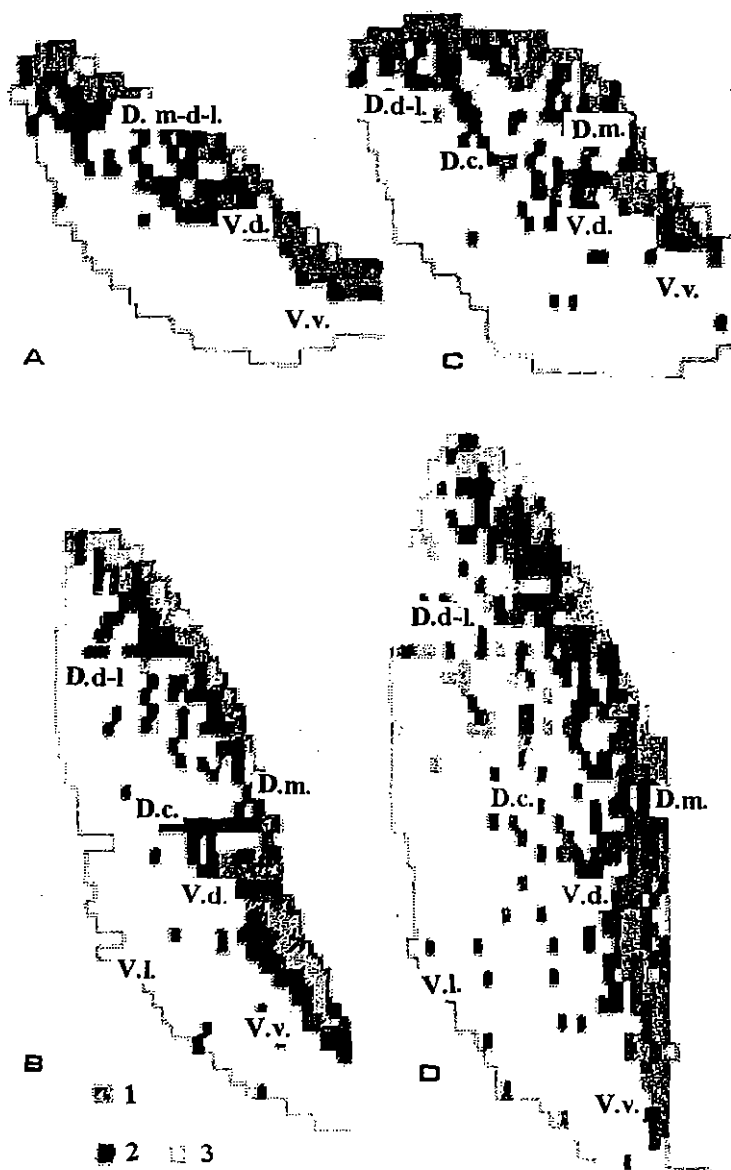
Figure 3. Computer diagrams of the same star sturgeon's brains made by automati imagination analysing system MAGYSCAN-2AR. 1,2,3 - zones of the maximum (1), middle (2) and minimum (3) cell density, other abbreviations as on figure 2.

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Tiny fishes (50-52 days old)

This stage of development is of the special importance for sturgeons as far as it is marked with releasing of the youth in the natural environment (Kasimov at al., 1986). Central nervous system on this age acquires the definite structure, stabilization of conditional reflectory reactions takes place. It should be noted that growing of the youth under different ecologic conditions results in development of the steady behaviour reactions, adequate to the environmental conditions (Kasimov, 1980). On the 50th to 52nd day A.H. in the "reservoir" youth the telencephalon features change insignificantly: the hemisphere square gets 570 ± 12 square units, the neurone sizws are given in the Table. The zonal differentiation of hemisphere has not completed yet. (Figure. 2B, 3B).

The '"pond " youth leave significantly behind the "reservoir " youth fishes in respect to growth and brain differentiation rates. Hemisphere square increases in size more than twice and gets 1250 ± 12 square units. Pallium and subpallium zones complete their differentiation, the level of their development is almost the same as in the mature brain (Figure 2D, 3D). Increasing of the average size of the neurones in the pallium zones results from the increase in number of large neurones themselves.

The obtained data coincide with the results of behaviour, biochemical and genetic studies in star sturgeon brain. So, it was shown that in the " pond " specimens even on the 30th to 35th days A.H. conditioned reflexes arise quicker, than in the " reservoir " fishes, skills exist longer and in their nervous tissue the more DNA/RNA concentrations are recorded. Later these differences become more pronounced (Nikonorov, Vitvitzkaya, 1991).

Thus, it has been shown that since the earliest stages of development, ecologic factors influence greatly on the formation of the CNS structuro-functional stereotype in the fishes. This is especially true for the " sensory starvation " under reservoir conditions, supressing the sturgeon CNS activity and , consequently, influencing the futher fortune of hutchlings after releasing in River.

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PHYSIOLOGICAL STRESS RESPONSES TO HANDLING IN

PADDLEFISH (*POLYODON SPATHULA*) WITH FRESHWATER AND

SALINE-WATER RECOVERY

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Introduction

Paddlefish (*Polyodon spathula*), a once abundant chondrostean fish of the Mississippi-Missouri River system, are an attractive species for aquaculture because of their high growth rate and delicate flesh (Kroll et al. 1994). Although the ecology and life history of the paddlefish have been studied to varying extents (Dillard et al. 1986; Reed 1992; Rosen and Hales 1982), the physiology of the paddlefish is minimally understood. For example, in a recent review, Barton and Iwama (1991) found very limited published information on corticosteroid stress responses in paddlefish. In this experiment, we investigated several physiological effects of handling. Also, as aquaculturists often use salts to improve survival and health of stressed fish (Wedemeyer, in press), we evaluated saline-water remediation of the stress response.

The objectives of our experiments were threefold. First we wished to determine both the magnitude and the time course of physiological responses to a brief handling stress. Second, we monitored the effect of adding salt to the recovery medium on these stress-induced changes. Finally, the fish were subjected to 1 h of chasing to determine the limits of their physiological stress responses. We measured changes in hematocrit, and plasma cortisol, glucose, and chloride in both experiments.

Methods and Materials

The juvenile paddlefish used in this experiment were hatched from eggs collected from wild adults, and were reared indoors at Gavins Point National Fish Hatchery. The fish were held in 1.8-m-diameter circular tanks containing 1320 L and having a flow-through of 39 L/min. For the handling experiment, the water supply (16°C) was pumped from Lewis and Clark Reservoir, passed through a drum filter, and disinfected with ultra-violet irradiation. For the chasing experiment, well water (13°C) at a flow-through of 21 L/min

was used. Fish were left undisturbed and unfed for 24 h preceding each experiment.

Handling Experiment with Salt Recovery. Juvenile paddlefish (mean weight, 157 ± 4.4 g) were removed from stock tanks, held in dip-nets for 30 s, and placed into tanks containing either fresh water (FW), or saline-water (SW, 0.5% noniodized NaCl). The tanks were well aerated by airstones delivering compressed air. The fish were divided into groups of 10-12 in separate tanks for sampling at 1, 3, 6, and 24 h so that sampling would not disturb the remaining fish. In addition, fish were sampled from the stock tanks at 0 h to provide prestress control values, and at 3, 6, and 24 h to provide resting-level control values.

Chasing Experiment. Paddlefish (187 ± 7.3 g each) in the two experimental tanks, which were located apart from the remaining two tanks, were subjected to continuous and vigorous chasing with intermittent dip-netting for 1 h. At 0, 1, 2, 4, and 24 h after the end of the chasing, five fish from each experimental tank were sampled (for $N = 10$). In addition, five fish were sampled from all tanks before chasing to provide prestress control values, and five fish were sampled from the control tanks at 4 and 24 h to provide resting-level control values; these tanks were "sham-sampled" (same sampling disturbance but without removing fish) at the other sample times.

Sampling and Analysis. To obtain blood samples, fish were anesthetized by immediately placing them in a lethal concentration (400 mg/L) of tricaine methanesulfonate (MS-222). This method has been shown to effectively arrest physiological stress responses in salmonids (Wedemeyer et al. 1990). Blood was collected from the caudal vasculature with a heparanized syringe and needle that was inserted ventrally immediately behind the anal fin (Houston 1990). Capillary tubes containing blood were centrifuged for 5 min, and the hematocrit was read as percent packed cell volume (% PCV). The remaining blood was centrifuged for 10 min, and the plasma was stored at -20°C for later analysis. Plasma glucose was determined by a spectrophotometric measurement of ortho-toluidine reaction (Wedemeyer et al. 1990). Plasma chloride was measured on a Corning Model 925 chloride analyzer using 20 μL samples. Plasma cortisol was measured in the unextracted plasma by radioimmunoassay (Foster and Dunn 1974). Standards were prepared by adding known amounts of cortisol (Sigma Chemicals, St. Louis, Missouri) to standard diluant made with bovine serum albumin. Data from all treatments were subjected to analyses of variance followed by Tukey's test ($P < 0.05$) to determine differences among means.

Results and Discussion

Response to 30-s Handling. After 30 s of handling, plasma cortisol significantly increased ($P < 0.05$) from 2.2 ± 0.6 to 11 ± 1.8 ng/mL after 1 h (Figure 1). The response was similar in pattern to that in teleosts, but was much smaller in magnitude (Figure 2) and was also lower than what has been previously documented in many teleost fishes from a variety of disturbances generally (Barton and Iwama 1991). Plasma glucose varied by treatment ($P = 0.015$), but no levels were conclusively greater than controls (Table 1). No significant change in either plasma chloride (mean level of 101 ± 0.6 mmol/L) ($P = 0.12$) or hematocrit ($P = 0.06$) was evident. Hematocrit data is presented in Table 1 for comparison to the 1-h chasing data.

Effect of Saline-water Mitigation. Saline water as a recovery medium did not significantly alter any physiological constituent measured. The mean plasma cortisol for saline-water recovery was slightly lowered, but not significantly so (Figure 1).
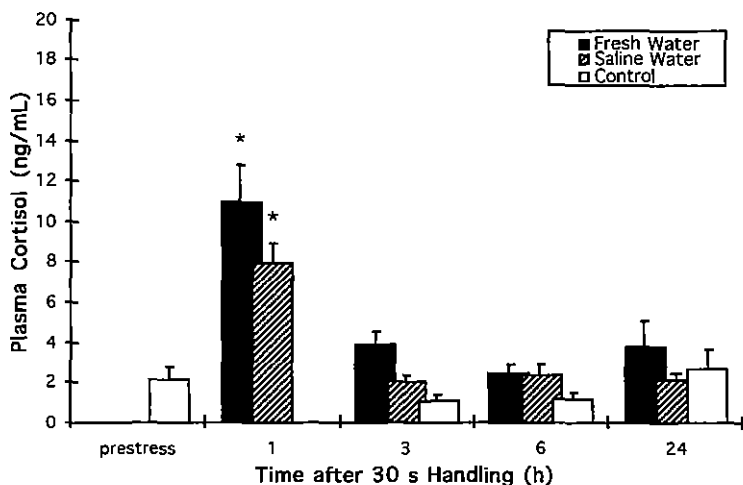
Figure 1. Mean + SE plasma cortisol in juvenile paddlefish subjected to 30 s of handling, with recovery in fresh water (FW) or water containing 0.5% salt (SW). The asterisks mark the values that are significantly different from the prestress value ($P < 0.05$).
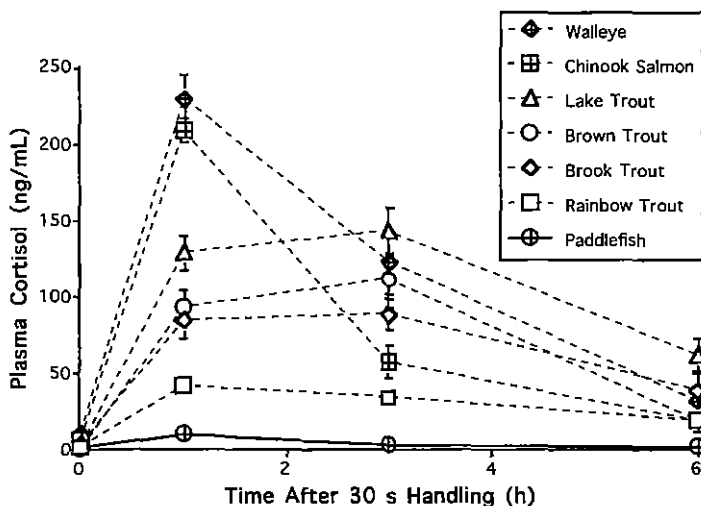
Figure 2. Examples of mean ± SE plasma cortisol levels in a variety of teleost fishes and paddlefish following 30 s of handling. Walleye (Barton and Zitzow 1995) and chinook salmon (Barton and Schreck 1987) had relatively high cortisol responses, while the trouts and chars (Barton, unpublished data) exhibited a range of responses. In contrast, paddlefish (solid line) showed a relatively low cortisol response.

Table 1. Mean ± SE plasma glucose and hematocrit in juvenile paddlefish recovering from a 30 s handling stress in fresh water (FW) and saline water (SW). Common letters mark treatments that are statistically indistinguishable ($P > 0.05$).

| Time after handling (h)	Glucose (mg/dL)				Hematocrit (% PCV)			
	FW		SW		FW		SW	
prestress	71 ± 6.5	a, b			22.8 ± 0.85	a		
1	63 ± 4.3	b	72 ± 4.5	a, b	26.5 ± 2.74	a	23.4 ± 2.58	a
3	66 ± 4.9	b	71 ± 4.6	a, b	27.7 ± 1.78	a	27.3 ± 1.12	a
6	78 ± 6.2	a, b	77 ± 3.2	a, b	23.9 ± 1.15	a	23.8 ± 0.80	a
24	92 ± 9.7	a	75 ± 3.4	a, b	25.5 ± 1.07	a	26.7 ± 1.21	a

Response to 1-h Chasing Stress. Plasma cortisol increased after 1 h of continuous chasing from 0.19 ± 0.106 to 13 ± 4.8 ng/mL cortisol ($P < 0.05$) (Figure 3). Because 1 h of vigorous chasing left the fish visibly exhausted, we speculate that these values approached the maximum plasma cortisol level attainable from acute stress in paddlefish. No significant differences were found among plasma glucose values. Plasma chloride levels, which averaged 104.7 ± 0.70 mmol/L, did not vary significantly throughout the study. Although ANOVA detected significant variation in the hematocrit data ($P < 0.0001$), Tukey's test found no specific differences (Table 2).

Table 2. Mean ± SE plasma glucose and hematocrit in juvenile paddlefish recovering from a 1-h chasing stressor in fresh water (FW) and saline water (SW).

Time after handling (h)	Glucose (mg/dL)		Hematocrit (% PCV)	
prestress	82 ± 7.7	a	24.3 ± 1.22	a
0	97 ± 9.5	a	26.2 ± 1.32	a
1	81 ± 2.9	a	not available	
3	87 ± 6.7	a	29.2 ± 1.34	a
4	73 ± 5.0	a	21.2 ± 1.62	a
24	83 ± 7.5	a	23.8 ± 1.42	a

In summary, paddlefish did not exhibit physiological responses to handling similar in magnitude to teleosts. The reasons for this difference are presently not known and investigations are currently in progress to assess differences in interrenal tissue structure between paddlefish and selected teleosts. Adding salt to the medium did not appear to significantly alter recovery time of the stressed fish. This is probably because the principal benefit of a saline recovery medium is to cushion osmoregulatory disturbances, which the paddlefish did not show.
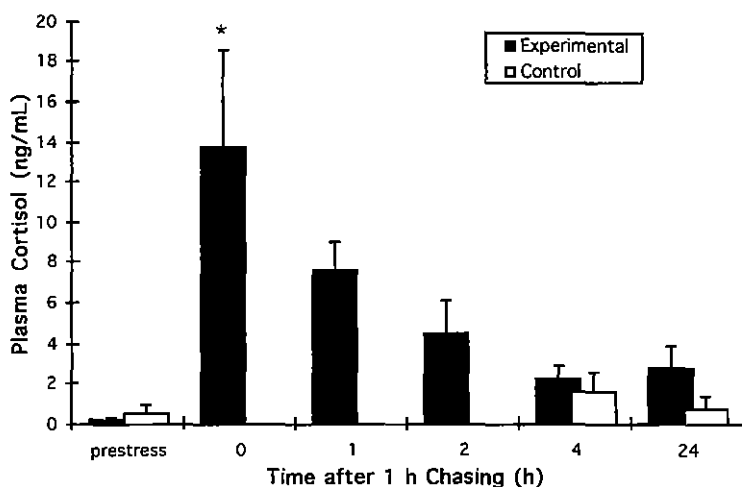
Figure 3. Mean + SE plasma cortisol in juvenile paddlefish after 1 h of chasing. The asterisk marks the bar which is different ($P < 0.05$) from the prestress value.

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Effects of Chlorinated Phenolics and Anti-Sapstain Agents (DDAC) on the Lethality and Swimming Performance of the Early Stages of White Sturgeon (*Acipenser transmontanus*)

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ABSTRACT

White sturgeon (*Acipenser transmontanus*) were spawned in California and fertilized or neurulated eggs were transported to B.C. In 1994, larvae and fry were exposed to 3,4,5,6 tetrachloroguaiacol (TeCG), 5 chloro-4-hydroxy-3-methoxybenzaldehyde (5VAN), 4 chloro-1,2-benzenediol (CAT) with concentrations between 0.001 and 10 ppm. The anti-sapstain didecyldimethylammonium chloride (DDAC) was also used in concentrations between 0.1 and 10 ppb. In 1995, larvae and fry were exposed to 4,5 dichloroguaiacol (DCG), 4,5 dichlorocatechol (DCAT), 6 monochlorovanillin (6VAN) with concentrations between 0.001 and 100 ppm. Pentachlorophenol was also used in concentrations between 0.001 and 1.0 ppm and DDAC in concentrations between 10 and 2000 ppb. In 1994, acute lethality tests were performed for 96 hr while in 1995 these tests were 24 hr in duration. The growth of test survivors was also measured. Swimming performance was measured on one group of fry after a 24 hr exposure of 1 ppm DCG and another group of fry after a 24 hr exposure of 1 ppm DCAT. There was a general trend to decreasing sensitivity with developmental age and increased mortality with handling. There was a significant difference in swimming performance after exposure to DCG and DCAT.

Keywords
Sturgeon; guaiacol; catechol; vanillin; phenol; anti-sapstain; acute; lethality; swimming performance.

INTRODUCTION

The Committee on the Status of Endangered Wildlife in Canada have classified the white sturgeon as vulnerable (Lane 1991). White sturgeon (Acipenser transmontanus) grow and spawn in the mainstem of the Fraser river. Water quality is of utmost importance to this large and long-lived

species. In the past many large adults have been found dead throughout the lower Fraser and there has been no recruitment in the upper Columbia River since the 1970's (Lane 1991). This has raised public concern about the fate of the species and the possibility that toxic effluents may be playing a role in their survival. Many authors have documented the detrimental effects of pulp mill effluents (Balk *et al*. 1993; Santos *et al*. 1990; Vijayram *et al* 1991; Van der Kraak 1992; Servos *et al* 1992) In addition adverse effects of anti-sapstain have also been documented (MacKinnon and Farrell 1992; Nikl and Farrell 1993; Johnston *et al*. 1996 in press). However, we are unaware of any toxicological studies on white sturgeon, especially the early life stages which are often the most sensitive (Rosenthal *et al*. 1976; McKim 1977; Burton *et al*. 1979; Hall *et al*. 1981).

Pulp and paper waste water discharge has increased and shifted the loadings to the Fraser river from 93% below Lytton prior to 1965 to 51% above Lytton in 1985 (Farrell 1993). White sturgeon most likely spawn in the main stem Fraser river above Mission therefore white sturgeon eggs and developing larvae are likely to encounter toxins from pulp mill waste water.

In view of this important knowledge gap, we used larvae and fry to test the acute and sublethal effects of chlorinated phenolics and an anti-sapstain, didecyldimethylammonium chloride (DDAC). These selected compounds have been detected in the Fraser river.

MATERIALS AND METHODS

Fish Supply and Testing

Approval was obtained on three separate occasions to transport eggs from gravid female white sturgeon in California because no gravid fish were available in B.C. In 1994 fish were spawned according to Conte et al. (1988) at " The Fishery" near Sacramento. Non-fertilized eggs, sperm and fertilized, de-adhesed eggs were transported in the dark to B.C. in plastic containers placed in insulated coolers maintained at 15^0 C $\pm 1^0$ C. Attempts to use non-fertilized eggs and sperm after transportation proved unsuccessful. Fertilized eggs however, were successful in transport with a mortality at hatch of 15%. In 1995, on separate occasions fertilized, de-adhesed eggs and neurulated eggs were successfully transported to B.C. from the University of California at Davis. Spawning and transport conditions for these eggs were similar to those in 1994 and mortality at hatch in 1995 was 5%.

Incubation, rearing and testing were done in quarantine at Malaspina University College (1994, 1995) or at Simon Fraser University (1995). Incubation, rearing and testing were done at 15^0 C $\pm$ 1^0 C with filtered, recycled water. Approximately 10% of the water in the re-circulation system was replaced every week. In 1994 and 1995 water hardness never exceeded 34.1 mg/l $CaCO_3$ and pH ranged from 6.5 to7.9. Water hardness was tested with a Hach kit and pH was tested with a Corning model 210 pH meter or a Fisher 910 pH meter. Effluent was treated with 50 ppm Cl_2 for 24hr, dechlorinated and then disposed of into municipal sewage.

After hatching the larvae were allowed to swim into a holding tank to minimize handling. Eggs, larvae and fry were taken from these stock populations and used in subsequent tests. Once the yolk sac was absorbed, food (Moore-Clark, 0.01 cm. trout diet) was presented to the fry for 15 min/hr for 12 hr in each of 24 hr period until most of the fish were eating the food. After 15 min uneaten food and feces was siphoned out of the tank. The developmental transition to feeding resulted in significant mortality ; a 40% mortality was incurred in 1994 and 15% in 1995. Six weeks after fertilization there is less than a 0.05% accumulated mortality per week.

In 1994, for each duplicate concentration of test chemicals 10 fry were allowed to acclimate to 78 L test aquaria for one week prior to testing. Larvae were tested in 38 L aquaria. Fry were exposed for 96 hr and larvae were exposed for 24 hr. Mortalities were removed at the end of the 24 hr exposure or daily during the 96 hr exposure. Isopropyl alcohol was used to dissolve the toxins.

In 1995, larvae or fry were placed into 1.5 L glass chambers for 24 hr. Mortalities were recorded after 24 hr. After the test exposure any survivors were placed in separate plexiglass rearing units supplied by a common filtered re-circulated water source.

The choice of concentrations and chemicals in 1994 was guided by concentrations found in sediments from 1991 to 1992 (M.O.E. unpublished data 1994). Concentrations were 10, 1, 0.1, 0.01 and 0.001 ppm for 3,4,5, 6 tetrachloro-2-methoxyphenol (TECG), 5-chloro-4-hydroxy-3-methoxybenzaldehyde (5VAN), and 4-chloro-1,2- benzenediol (CAT) (Helix Biotechnologies, Richmond B.C.). The test concentrations for didecyl -n,n-dimethylammonium chloride (DDAC) (Lonsa, New Jersey) were 10, 1.0, 0.5 and 0.1 ppb. A stock solution of 10 ppm or 10 ppb was used to make serial dilutions for the remaining concentrations.

In 1995, the choice of concentrations and chemicals were guided by concentrations found in sediments and waste water (DOE data 1995, unpubl.) and by results in 1994. Concentrations of 4,5 dichlorocatechol (DCAT) and 4,5 dichloroguaiacol (DCG) were 10, 1.0, 0.1, 0.01, 0.001 ppm for larvae and fry. Whereas, the concentrations of 6 monochlorovanillin (6VAN) for larvae were 10, 1.0, 0.1, 0.001 ppm and 100, 50, 10, 1.0, 0.1 ppm for fry. The concentrations of DDAC used with larvae were 1.0, 0.5, 0.1, 0.05 ppb and 2000, 1000, 100 and 10 ppb for fry. The concentrations of pentachlorophenol (Dr. F. Law, S.F.U.) used on fry were 1.0, 0.5, 0.1, 0.01 and 0.001 ppm. MVAN, DCAT, and DCG were purchased from Helix Biotechnologies (Richmond, B.C.).

Swimming Performance Tests

In 1995, preliminary studies were performed on 45 day old fry. The average weight at the start of the tests was 1.0 g $\pm$ 0.3 g and average total length was 6.6 cm $\pm$ 0.7 cm. The stock population was fed to satiation with Biodiet (Bioproduct Inc., Buhl Id.).

U_{crit} was determined for 2 groups of 10 fry 4-12 hr after being individually placed in a swim chamber. Recovery for 72 hr and then exposure to a single concentration (1 ppm) of either DCG or DCC for 24 hr. Following chemical exposure they were allowed 72 hr to recover prior to repeating the U_{crit} measurement. U_{crit} was determined according to Brett (1964). After the 4-12 hr acclimation period flow was increased at 3 cm/s in 20 min increments until fatigue occurred. The outflow grid wasn't electrified. Therefore, to discern fatigue from laziness water velocity was decreased slightly until the fish re-oriented itself into the current and then the flow was increased to the previous rate until fatigue. A fatigued fish would not re-orient itself after the flow was adjusted three times. One quarter of the chamber was not covered with black plastic to view the exhausted fish and to provide some stimulation to swim away from the lighted area. After fatigue the fish were allowed to recover for 40 min and then anaesthetized with buffered MS 222, before being weighed and measured. The inside dimensions of the swim chamber are 24.0 cm. x 15.5 cm. x 24.5 cm. The propellor was driven by a permanent magnet D.C. motor and controlled via rheostat and tachometer. Water velocity was calibrated with a Swoffer instrument model 2100 series current velocity meter (Seattle, Wa.). Freshwater was supplied from the recirculation system to the inner swim chamber at a rate of 1L per minute and allowed to flow into the outer jacket where it was directed to the out flow drain. This rate was maintianed throughout the performance tests. During the acclimation period a flow of 1/4 body length per second (BL/s) was maintained. The outflow oxygen did not fall below 6 mg/l O_2 as measured by a

hand held oxygen meter (Oxyguard Inc.). Temperature was maintained at 17° C throughout the tests.

RESULTS

All results for acute toxicity tests are presented in table 1.

Toxin Exposures

TeCG was tested at concentrations from 0.001 to 10.0 ppm. The LC_{50} for larvae and fry is between 1.0 and 10.0 ppm.

DCG was tested at concentrations from 0.001 to 10.0 ppm. The LC_{50} for larvae was less than 0.001 ppm and between 0.1 and 10 ppm for fry.

5VAN and 6VAN was tested at concentrations from 0.001 to 10.0 ppm. The LC_{50} for larvae and fry tested with 5VAN was between 1.0 and 10.0 ppm. The LC_{50} for larvae tested with 6VAN is below 0.001 ppm and between 0.001 and 0.01 ppm for fry. Survivors exhibited a high degree of morbidity during the test with both types of vanillin. After the test the survivors recuperated once they were moved into clean water.

DCC was tested at concentrations between 0.001 and 10 ppm. The LC_{50} for larvae was 0.01 ppm and 0.51 ppm for fry.

Pentachlorophenol was tested at concentrations between 0.001 and 1.0 ppm. The LC_{50} for fry is between 0.01 and 0.5 ppm.

In 1994 DDAC was tested at concentrations between .0001 and 0.01 ppm. The LC_{50} for larvae was 0.00112 ppm and between 0.001 and 0.01 for fry. In 1995 DDAC was tested at concentrations between 0.00005 and 2.0 ppm. The LC_{50} for fry is between 0.001 and 0.01 ppm. Mortalities occured instantly and exhibited a discoloration of the skin from grey to white.

The solvent controls for the larval tests exhibited high mortalityin 1994. Therefore, in subsequent tests in 1994 and 1995 the solvent was changed from isopropyl alcohol to ethyl alcohol. There were no mortalities in later solvent controls after this change was made. Also, larvae or fry exhibited little or no mortality in normal controls in 1994 or 1995.

Growth Tests

The attempt to follow growth rates post-exposure was largely unsuccessful. There was an increase in mortality after each weekly weighing. Multiple stressors (transport,toxin exposure, weighing) may have a cumulative mortality effect on sturgeon fry.

Swimming Performance
There was a significant ($p < 0.05$, paired Student t test) decrease in swimming performance after exposure to 1 ppm 4,5 dichlorocatechol and 1 ppm 4,5 dichloroguaiacol.

DISCUSSION
Recent evidence about the timing and location of mature individuals and spawning pairs in the Fraser river is largely anecdotal (Roseneau, pers. comm., 1995). White sturgeon are thought to

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broadcast spawn once temperatures reach 10 to 15^0 C (Scott and Crossman 1973) and at these temperatures hatch in 7 - 10 days (Conte *et al* 1988). Two days after hatching the larvae are 13.0 mm long, active but swim poorly (Conte *et al* 1988). Spawning in the Fraser is thought to occur generally between Lytton and Mission during the months of April to June when water temperatures reach the desired level. The distance and rate of egg or larval dispersion is unknown. Therefore, it is also unknown how much water, suspended sediments or bottom sediments affect eggs, larvae or fry. Sekela *et al*. (1994) found that 6 monochlorovanillin had a concentration of 11.1 ng/g in dry weight suspended sediments (March 30) versus 1.359 ng/l in water on the same day. There is a general trend of decreased sensitivity with developmental age to the chlorinated phenolics and DDAC tested. The age and location of sturgeon is important in determing their exposure to toxins.

Sturgeon populations have been threatened or lost by anthropogenic factors in various river systems in the world. They are a long lived and valued species of the Fraser river system. More information is needed on their ecology and life history within the Fraser river before more relevant work can be done.

CONCLUSION

The acute toxicity testing of larvae and fry was a success. However, alternative test methods are needed for growth studies to reduce handling stress. Multiple stressors may contribute to the cumulative mortality of sturgeon. Sublethal exposure of DCC and DCG affects the swimming performance of sturgeon fry.

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Table 1. Acute toxicity results for 1994 and 1995. Probit analysis was done (95% confidence limits) where data permitted. LC_0 is the highest concentration at which there were no mortalities. LC_{50} is the concentration or range of concentrations where there was 50% mortality. LC_{100} is the lowest concentration at which 100% mortality occurred.

Table 1:   Acute Toxicity Results

1994 (96HR) | 1995 (24HR)

3,4,5,6 TETRACHLOROCUAIACOL (ppm) | 4,5 DICHLOROGUAIACOL (ppm)

	LC_0	LC_{50}	LC_{100}		LC_0	LC_{50}	LC_{100}
LARVAE	1.00	1.0-10	10.00		<0.001	<0.001-0.01	0.01
FRY	1.00	1.0-10	10.00		0.1-1.0	0.1-10	10.00

5,MONOCHLOROVANILLIN (ppm) | 6,MONOCHLOROVANILLIN (ppm)

	LC_0	LC_{50}	LC_{100}		LC_0	LC_{50}	LC_{100}
LARVAE	1.00	1.0-10	10.00		<.0.001		0.001
FRY	0.5	0.5-5	5.00		0.1-1.0	0.1-10	0.1-10

DIDECYLDIMETHYLAMMONIUM CHLORIDE (ppm) | DIDECYLDIMETHYLAMMONIUM CHLORIDE (ppm)

	LC_0	LC_{50}	LC_{100}		LC_0	LC_{50}	LC_{100}
LARVAE	<0.00001	0.00112	0.0001				
FRY	<0.00001	0.001-0.01	0.01		0.00001	0.001-0.01	0.01

4,5 DICHLOROCATECHOL (ppm)

	LC_0	LC_{50}	LC_{100}
LARVAE	<.001	0.01*	0.1
FRY	0.01	0.51*	10.00

PENTACHLOROPHENOL (ppm)

	LC_0	LC_{50}	LC_{100}
FRY	0.1	0.01-0.5	0.5

*=probit analyses,95% confidence limits

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THE OOGENESIS INHIBITION, STEROIDOGENESIS AND MORPHOMETRIC

STUDIES OF THE HYPOTHALAMO-HYPOPHYSAL SYSTEM IN THE RUSSIAN

STURGEON EXPOSED TO LOW ENVIRONMENTAL pH

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Abstract: Russian sturgeon juveniles, 5.5-6.5 months old, were bred at pH 4.0-4.8. By the end of the experiment in the treatment fishes the body-weight became 13% less than the initial one, while in control fishes it grew 16% more. In treatment females the sex cells fund grew more, but the sex differentiation process retarded in its initiation. The statistically reliable rise in the testosterone-plasma content was recorded in the treatment fishes at the beginning of the acidification exposition, and in control females - during the cytological differentiation. No sexual specificity in the oestradiol-17β content was found. The weighed amount (%) of the hypothalamus nucleus preopticus's granulated neurosecretory cells and of the hypophysis's gonadotropocytes appeared to be higher in the treatment fishes at the beginning of the acidification exposition, and in all the females during all the experiment. It is reasonable to be attributed to the development of an adaptational syndrome, and in females to the sex differentiation period.

Introduction

It is well known that water acidification causes inhibition of fish's reproductive activity (Sangalang, 1990; Tam et al., 1990) a fall in their abundance and a consequent decrease in the fish's' species diversity (Beamish and Harvey, 1976). When at low pH, reproductive stability depends on gonad development success during its most critical stages, if extreme effects are considered. The period of sex differentiation appears to be one of such stages; it is pre-determined genetically, but could be easily affected by the environment. Since the development rate in sturgeon juveniles is rather low, investigators have a chance to provide experiments at an exact stage of early gametogenesis. This

paper's goal was to study the effects produced by highly acid water on gonad development and the steroidogenesis processes, and on the nucleus preopticus and hypophysis in Russian sturgeon juveniles, while anatomical and cytological differentiation takes place. In rrecent years, the water acidity effect on Caspian Sea sturgeon's natural reproduction has become more and more pronounced.

Materials and Methods

Russian sturgeon (*Acipenser guldenstadti* Brand t) juveniles (30-120 g) were used in the experiment. At the age of 5.5 months, some fish were removed for 25 days from water with pH 7.2-7.6 to water with pH 4.0-4.8. Water properties were: hardness - 11 mg/L, alkalinity (as HCO_3^-) - 3.1 mg/L, temperature 18.5-20.0°C, O_2 - 7.6-9.6 mg/L, NH_4 - 0.20-0.56 mg/L.

To investigate the effect of acidification on the reproductive system, the gonads, brain and hypophysis of either the treatment or the control fish, they were fixed with the Bouin's Liquid and sealed with paraffin-wax. Sets of gonad slices were stained with Heidengain's hematoxyllin. Sets of hypothalamus and hypophysis slices were stained with GomoriGabu's paraldegid-fucsin (PAF) and azocarmine. Some sets of the hypophysis slices were stained using Herlant's method (Herlant, 1960). This method allows the possibility to differentiate PAF+ tireothropic and alcian-positive gonadotropic basophils. The area of the gonad frontal slice was measuredand the number of sex cells per slice were recorded. The nuclei mean diameter, the weighed amount (%) of the granulated neurosecretory cells in the hypothalamus nucleus preopticus and the number of gonadotropocytes in the adenohypophysis proximal *pars distalis* ventral region were estimated. Blood testosterone and oestradiol-17βcontent in the plasma were estimated using a radio immunological method (Morozov et al., 1988). All results are reported as mean plus standard deviation. Student's t-test was used to test for significant differences between treatment and control groups.

Results

Gonad condition

Water acidification produced a pronounced stress-like effect on the sturgeon juveniles. During the exposure period, food consumption decreased strongly and the fish's weight became 13% less, compared with the initial values, while the control fish's weight grew 16%.

After the 8-day exposure the goniums only were observed in gonads; the fat, stromal and generative parts were quite discrete in gonads (Figure 1.2). With the germinative epithelium being curved inside the ovary's generative part (specific form for sturgeon) and the goniums being solidly arranged (Figure 1.3), one could easily determine the fish's sex. Future males, on the contrary, had no furrow and the goniums were homogeneously distributed within the testicle's generative part (Figure 2.4). At this stage the gonad's mean frontal slice area varied greatly and the area of the generative part of gonads in females was much greater than in males (Table 1). The goniums' number per gonad generative area grew in all the treatment fish, both females (p<0.01) and males.
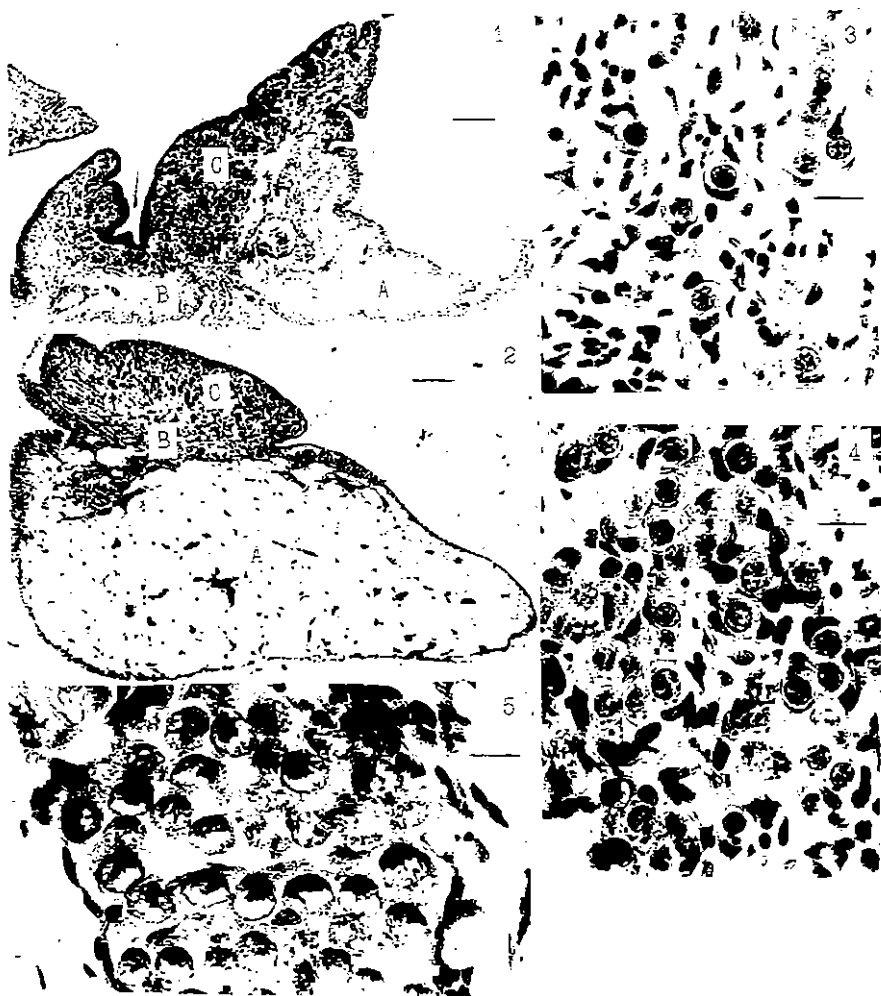
Figure 1-5. In the gonads of the Russian sturgeon the following
discrete parts (1-2) are well distinguished: fat part (A), so-
matic (B), generative (C). One may distinguish ovaries (1) by
the germinative epithelium, being curved insides the gonad (→)
and the sexual cells (3) being solidly arranged. In testicles
(2) goniums are distributed gomogeniously through its genera-
tive part (4). In three of eight control females at the end of
the experiment the oocytes were recorded (5). Fig. 1,2 - scale
bar 0,1 mm; Fig. 3-5 - scale bar 0,02 mm.

Table. 1. Gonad condition of Russian sturgeon control © and treatment (T) groups at 8 and 25 days in an acid environment beginning from 5.5 month old. Results are reported as mean+SD, lim. and CV. M=male, F=female

Days	C T	F M	Number of fish	Weight (g)	(1)	(2)	(1)/(2) (%)	(3)
8	C	F	11	66.5+6.8 (29-99) V=33.9%	16.0+2.2 (4.6-26.83) V=43.7%	4.2+0.6 (0.6-7.6) V=52.3%	25.7+2.4 (13.0-38.4) V=30.3%	10.8+0.9 (7.1-15.4) V=28.9%
8	C	M	12	84.9+8.2 (47-120) V=30.8%	24.3+6.6 (2.7-62.0) V=86.0%	1.9+0.3 (0.6-3.5) V=51.5%	13.9+2.4 (2.3-25.0) V=55.3%	11.7+1.2 (5.8-17.1) V=32.4%
8	T	F	7	66.7+8.3 (54-101) V=32.9%	15.1+2.9 (6.0-26.1) V=52.3%	3.5+0.6 (1.1-5.5) V=48.5%	23.9+3.2 (17.6-42.4) V=35.5%	15.1+0.6 (12.8-17.2) V=10.5%
8	T	M	18	72.0+4.7 (42-112) V=27.7%	15.2+1.7 (2.8-31.2) V=46.7%	1.3+0.2 (0.4-3.3) V=57.5%	11.9+2.3 (2.2-34.7) V=79.8%	13.0+1.1 (3.3-20.0) V=36.1%
25	C	M	8	82.8+9.5 (45-120) V=32.3%	30.2+2.9 (21.7-44.9) V=27.1%	6.6+1.2 (2.0-12.3) V=51.5%	21.1+2.7 (8.2-33.8) V=36.9%	15.3+2.1 (7.6-26.6) V=39.8%
25	C	M	11	105.3+11.2 (41-115) V=35.4%	24.0+3.3 (7.5-41.9) V=41.6%	1.5+0.2 (0.5-2.7) V=46.6%	9.1+2.1 (2.5-26.0) V=74.7%	11.4+1.7 (3.3-24.7) V=49.1%
25	T	F	8	72.0+7.0 (44-107) V=30.0%	15.2+2.2 (8.0-23.9) V=38.8%	2.5+0.3 (1.1-4.2) V=40.0%	15.8+2.5 (2.4-28.7) V=46.2%	14.6+1.6 (9.4-22.0) V=32.8%
25	T	M	12	66.5+4.8 (41-100) V=25.1%	15.4+3.3 (5.2-34.1) V=68.8%	1.2+0.2 (0.4-2.5) V=58.3%	9.6+1.4 (2.5-17.8) V=48.9%	14.6+1.9 (6.2-30.7) V=45.2%

(1) - Area of frontal gonad slice, mean*10^{-2}+SD*10^{-2}mm^2

(2) - Area of frontal generative part gonad slice

(3) - Number sex cells in 0.01 mm^2 of generative part area

After the 25-day exposure, all the control fish showed completion of anatomical differentiation of gonads, and three of eight females not only had oogonia, but oocytes in their ovaries (Figure 5). Once the cytological sex differentiation in females began, the ovary's and their generative slice-areas grew much more, as did the number of sexual cells per ovary area.

Treatment fishes had much smaller gonads, and the ovary generative slice areas in was significantly smaller, to a significance measurement of p<0.01. The described pattern of ovary anatomical differentiation occurred in 12 fish out of 20. The sex cells in all fish were represented by gonia only. Both in the control or experimental groups, the mean number of sex cells per gonad area was similar in males and females.

Plasma oestradiol-17B content

In most of the studied sturgeons (83%), the concentration of oestradiol-17β in the blood plasma varied within the range of 90-205 pg/mL. In 11 fish specimens only (14%), it varied in the range of 38-85 pg/mL. In two control fish the hormone content was much higher 339-375 pg/mL. If the fish from different groups were compared (Figure 6), no sexual or acidity-related specificity in oestradiol content was recorded.

Plasma testosterone content

In most of the studied sturgeon juveniles (75%), the concentration of testosterone in the blood plasma varied from 0.43 to 2.0 ng/mL. In 14 specimens (18%) the higher testosterone content from 2.19 to 5.6 ng/ml was observed, in 5 specimens the lowest values (0.07-0.37 ng/mL) were recorded. It is noteworthy that 12 of 13 specimens with the highest testosterone content were controls.

On the 8th day of exposure, the treatment fish, whether males (p<0.05) or females (p<0.05), had testosterone content significantly higher than the controls. On the 25th day, the hormone concentration in the blood of treatment sturgeon of both sexes was practically the same, but was higher in the control females. Undoubtedly this was because of the beginning of cytological differentiation and of the growth in sex cell number.

Nucleus preopticus

Investigation of the most differentiated and large-celled region of the hypothalamus nucleus preopticus revealed that the morphofunctional characteristics of the neurosecretory cells (NSC) were quite variable. For instance, an intra-individual variation in the mean diameter of their nuclei (7.7-9.6 um) and the PAF-index of the neurosecretory granules in their cytoplasm was somehow limited. The NSC were more or less similarly solidly arranged, the neurosecretory matter content in axons did not vary much, the broadness of the capillary system was usually the same and the picnomorphic cell number did not vary much. The weighed amount of NSC appeared to be highly variable (4-48%). The treatment juveniles on the 8th day of the experiment appeared to have much more granulated NSC (13.1±5.4), than the controls (5.2±2.7).

Adenohypophysis

When investigating the adenohypophysis gonadotropic region, we saw a wide range of
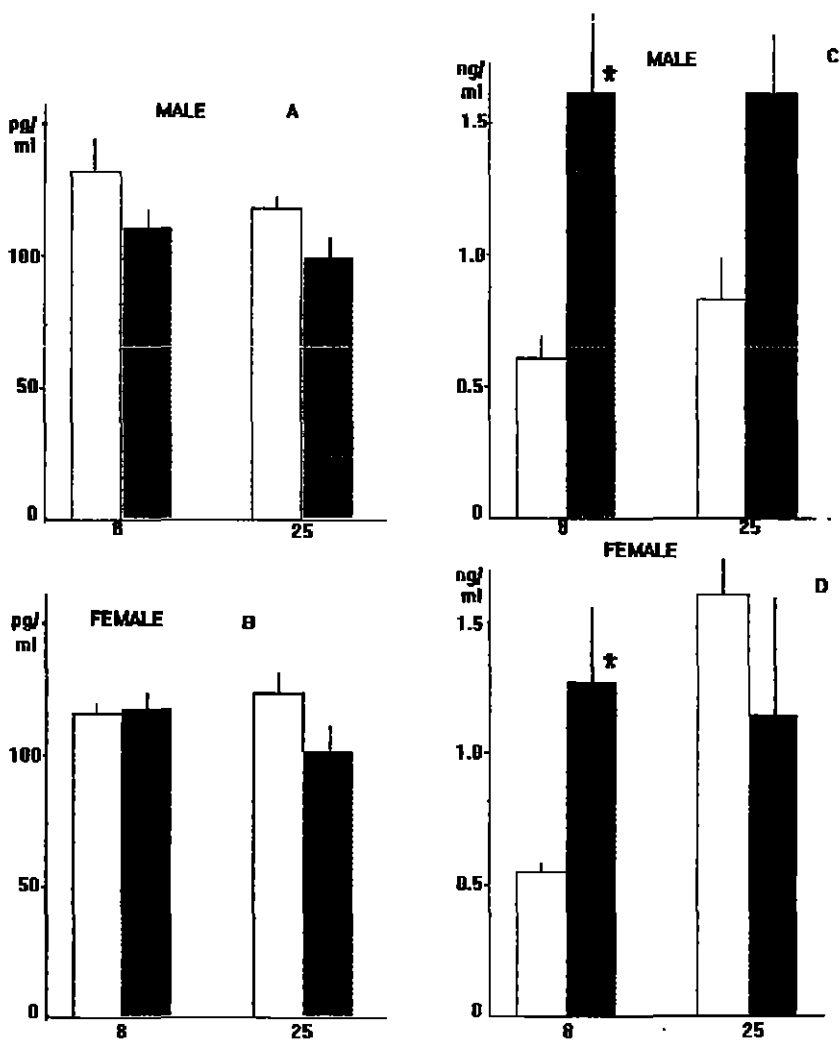
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Figure 6. Plasma oestradiol-17. (A and B) and testosterone (C and D) concentration. The fish were maintained at either pH 7.2-7.6 (open histograms) or 4.0-4.8 (hatched histograms). * indicates treatment group means significantly different from its respective control group. 176

morphofunctional states: the glandular parenchyma's cell differentiation, the progressive succession of states of gonadotropocytes, more and more active hormone production,and the most active holocrine secretion (Figure 7).

The gonadotropic region's early stages proceeded in the following way: a thin layer of epithelium cells, lying ventrally off the hypophysis's cavity, folds up; its cells are enriched with the cytoplasm; the secretory granules are stocked in them (these granules are stained with alcian blue better than with PAF+). The fish with indifferent gonadal glands or with ones at early stages of the sex differentiation process had cells with poorly granulated cytoplasm. The amount of granulated cells was low (2-11%) and they lay ventral-cranially up to the rostral *pars distalis*. If optically transparent vacuoles appeared in strongly granulated cells, it could be regarded as an evidence of their secretory activation. The stronger the secretory activity grew, the more expressed the holocrine cell-degeneration and cell-desolation processes were and more broad cavities appeared instead of the dead gonadotropocytes. During the period of

Figure 7. The adenohypophys's gonadotropic area in the Russian sturgeon. Conglomerates of the PAF+ colloid during an intensive secretion process. scale bar - 0.02 mm

highest secretory activity, a conglomerations of acidophilic and PAF+ colloids were found in these cavities. Similar colloid drops were observed in some fish in the boundary cavities between the neurohypophysis and *pars intermedium*, and between the epithelial folds in the dorsal part of the proximal *pars distalis*. A pronounced vascularization of the gonadotropical region and neurohypophys was observed at the same time.

The adenohypophysis gonadotropical region was recorded to be more active in females, especially if cytological sex differentiation had begun.Statistical analysis showed the marked sex specificity in the granulation rate of the gonadotropical cells. As it took place, the females had 2.0-56.6% (18.3$\pm$4.5%) cells granulated, and males had 1.6-29.3% (8.8$\pm$1.7%; p<0.05) . At low pH, treatment fish had a clear sign of early gametogenesis being stimulated, and the mean amount of granulated gonadotropocytes in them was higher (12.4$\pm$4.5%), than in the controls (4.3+1.0%).

Discussion

According to the analysis of the individual data, in sturgeon juveniles, the correlation between the characteristics of the gonadal gland development stage, the nucleus preopticus and hypophysis secretory activity was not complete. There were some fish of the same sex and at the similar gonadal

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development stage, but quite different in the amount of granulated neurosecretory cells in the nucleus preopticus, in the number of adenohypophysis gonadotropocytes, and in the content of the sex steroid hormones in blood. This might be attributed to the phase character of the secretory activity and to the polyfunctional character of the endocrine regulation in these cases. That is why we gave up with the analysis of the individual characters and preferred to analyze the group characters.

According to the experimental data, in juvenile Russian sturgeon females at low pH, the amount of sex cells increases. It might be caused by a more or less increased gametogenesis rate, as was observed in the treatment fish together with a significant rise in the serum testosterone content and increased amount of granulated neurosecretory cells and gonadotropocytes. It is well known that in fish at the early development stages, the overall androgen blood content is much higher than the oestrogen one. This situation is usually attributed to androgen regulating the growth (somatic) and morphogenetic processes, which are anabolic by their nature. At the beginning of the acid exposure, a rise in the testesterone content might proceed together with an intensified synthesis of some other steroid hormones (i.e. cortisol) and might be attributed to its role in homeostasis during stress conditions. At the same time, testosterone is well known for its gonadotropic activity (Giellen, Goose, 1984), and its ability to promote fish gametogenesis in early stages (Crim, Evanse, 1983). It should not be omitted that it was testosterone that was responsible for the higher oogenesis rate.

The short-term oogenesis promotion in rainbow trout, with respect to the acidification effect was described recently (Zelennikov, 1996). No changes in the male gonads were recorded. The fact that stress caused the rise in testosterone concentration, and consequently the gametogenesis promotion in females could only be attributed to the their gametogenesis rate (being initially higher than in males) to the much earlier sex differentiation, and therefore to a higher hormonal competence of the female sex glands. A high correlation index between blood testosterone concentration and the number of sex cells per gonad area might be evidence of testosterone's possible gonadotropic character. It should be noted that in the period when the anatomical sex differentiation was completed, and the sex-cell differentiation was initiated, the endogenous increase in testosterone concentration occurred in the control females on the 25th day of the experiment. The time coincidence between the periods of gonadotropocytes' granulation, their secretory activity, the increased testosterone content and the intensive gameto and gonadogenesis, observed either in treatment or control females, suggests that these correlations might be of a functional nature. As was described earlier, adenohypophysis activity is intensified during the period of sex cell differentiation (Fiodorov, 1989). The other data on the correlation of the timing of the steroid synthesis initiation in gonads and of the granulated cell's emergence in adenobypophysis in other fish species are rather contradictory (Van den Hurk, 1974; Van den Hurk et al., 1982). Nevertheless the presented data on Russian sturgeon juveniles does not seem to be in conflict with Jamamto's concept (1969) that the steroid hormones are the first to induce sex differentiation in fishes, but shows evidence of testosterone's special role in this process. On the contarary, oestradiol obviously does not significantly regulate the process of meiosis initiation in oogonia. Oestradiol's content in female blood was stable throughout the experiment and remained stable in the fish under stress.

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SEX STEROID LEVELS IN COLUMBIA RIVER WHITE STURGEON

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Successful management of white sturgeon (*Acipenser transmontanus*) in the Columbia Basin requires increased efforts to understand the reproductive health of sturgeon populations. As the populations of chinook salmon have declined in the Columbia forcing greater restrictions on harvest, interest in recreational sturgeon fishing has soared, presenting state and federal agencies with a challenge to understand basic reproductive parameters in this species in order to better estimate the productivity, potential yield, number of broodstock, reproductive success in different parts of the basin, and the effectiveness of transfer programs. Efforts have been made to assess maturity and identify sex in wild white sturgeon. Currently, biologists perform surgery on sturgeon sampled in the field to examine the gonads for determination of sex and estimation of reproductive status. This procedure is difficult to perform and the accuracy of the results is limited due to the very small gonads in non-reproductively active individuals, which can lead to misidentification of the sex or biopsying the wrong tissue. A simple, fast procedure for determining sex and maturity of white sturgeon would benefit agency biologists by allowing for a more accurate assessment of population status and future reproductive potential of white sturgeon. The objective of this study was to determine if plasma sex steroid levels can be used for distinguishing between sexes and maturity status in wild white sturgeon.

Adult white sturgeon were sampled after harvest near Astoria, Oregon in February 1996. From each of 31 individuals, blood was sampled for plasma preparation and the sex identified by examination of the gonads. Plasma levels of testosterone (T), 11-ketotestosterone (KT), and estradiol (E2) were assayed by radioimmunoassay. Plasma levels of T or KT greater than 3 ng/ml were only found in males; whereas levels less than 3 ng/ml were found mostly (but not exclusively) in females (Fig. 1). Levels of E2 were low in all fish samples, regardless of sex.

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